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Seed Treatment of Field Crops with Systemic Insecticides[1]

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A number of entomological workers in recent years have been investigating the practicability of applying systemic insecticides in various manners at planting time in order to protect seedling plants from insect and spider mite damage. Successful pest control on small seedling plants has often presented difficulties in the past. There are several reasons for this, notably, susceptibility to pest attack at this stage of growth, little plant surface to receive and retain insecticidal deposits, and the rapid rate of growth of small plants, which makes it difficult to attain more than initial mortalities when conventional insecticides are used. Experimental results obtained by applying systemic insecticides at planting time show such promise in overcoming these difficulties that it now appears that these methods of treatment will soon become recommended practices on a number of crops.

Although a great deal of experimental work is currently being done with systemic materials applied at planting time, only a few published papers have appeared to date. Ripper *et al.* (1950), Ivy *et al.* (1950), Reynolds *et al.* (1953), David & Gardiner (1955), and a few others, have shown that systemics are absorbed into seeds in sufficient quantities to give insecticidal action. Much of the early work was with schradan and demeton,[2] although several other materials, particularly dimefox and para-oxon, also have been investigated. Most reports have been concerned with seed soaks or modifications wherein systemic solutions have been applied in various ways at time of seeding. Ivy (1952) reported that activated charcoal was a very satisfactory carrier for coating seeds, in view of the fact that larger amounts of material could be used with little phytotoxicity. Recently, Kantack (1955) discussed the use of Carbowax as a carrier.

Schradan and demeton are effective on a rather narrow range of pests, namely, on spider mites, aphids, mealybugs, and a few others. Ivy *et al.* (1954) and Clark *et al.* (1955) reported that several new systemic insecticides, Am. Cyanamid 12008 (*O,O*-diethyl *S*-(isopropylmercapto)methyl phosphorodithioate), Am. Cyanamid 12009 (*O,O*-diethyl *S*-(n-propylmercapto)methyl phosphorodithioate), and Thimet, were effective on a much wider range of insect pests when applied at planting time on cotton.

It has been demonstrated by Fukuto *et al.* (1955, 1956), Metcalf *et al.* (1955), and March *et al.* (1955) that the compounds *O,O*-diethyl *O*-2-(ethylmercapto)ethyl phosphorothionate (Systox thiono isomer) and *O,O*-diethyl *S*-2-(ethylmercapto)ethyl phosphorothiolate (Systox thiol isomer) are metabolized in plant and animal tissues to the corresponding sulfoxide and sulfone derivatives which are the ultimate systemic toxicants. The newly developed organo-phosphorus insecticide, Bayer 19639 (*O,O*-diethyl *S*-2-(ethylmercapto)ethyl phosphorodithioate)[3] is a structural relative of the two Systox isomers, and Thimet is the closely related compound, *O,O*-diethyl *S*-(ethylmercapto)methyl phosphorodithioate. Recently the plant metabolism of Bayer 19639 and of Thimet was investigated as well, using P^{32} radiotracers (Metcalf *et al.* 1957). In these studies it was found that Bayer 19639 and Thimet behave in a completely analogous fashion (although metabolism takes place at somewhat different rates) and that the process is primarily oxidative.

In the case of Bayer 19639 in cotton, lemon, bean, and alfalfa plants, oxidation proceeds rapidly to the sulfoxide *O,O*-diethyl *S*-2-(ethylsulfinyl)ethyl phosphorodithioate, and more slowly to the corresponding sulfone, *O,O*-diethyl *S*-2-(ethylsulfonyl)ethyl phosphorodithioate. In both the sulfoxide and the sulfone compounds the phosphorodithioate is further oxidized to phosphorothiolate. Interestingly enough, the parent compound is oxidized almost immediately after absorption, so that at intervals of a few days to a month after application, all four oxidation products may be present in the plant residues, but with no parent compound.

Since 1954, research in southern California has been in progress with these and other systemic compounds. Most of this work has involved the more important field crops, such as alfalfa, cotton, and sugar beets. Results obtained to date in radiotracer studies of systemic insecticide distribution within the plant, in field experiments, and in residue studies are herein reported.

Radiotracer Studies.—After initial laboratory and field studies established the fact that Bayer 19639, Thimet, and the thiol isomer of Systox were toxic to certain insects and spider mites, it was found that little or no information on the distribution of the toxicants in plants and the magnitude of residues following various

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[2] Referred to as Systox in previous papers from this Station.
[3] Produced by Farbenfabriken Bayer and referred to as dithio-Systox in earlier papers from this Station; also called Di-Syston.

methods of treatment was available. It was decided to resolve at least some of these problems with radiotracer techniques.

The P^{32} labeled Bayer 19639, Thimet, and thiol isomer of Systox were prepared by methods described by Fukuto *et al.* (1955) and Metcalf *et al.* (1957). In the experiments designed to compare the relative efficiencies of various methods of treatment, three formulations of these materials were prepared: (1) 50% compound on activated charcoal, (2) 25% compound as an emulsion concentrate with 70% xylene and 5% Atlox 1045A, and (3) 1% compound on 30/40 mesh granular Attaclay.

Each of these formulations was applied to cotton, alfalfa, and sugar-beet seed at 1% of the active material per seed weight (about 25% of the dosage used in the field). The charcoal formulations were applied dry to mechanically delinted cotton seed, and as a slurry to the alfalfa and sugar-beet seeds. The emulsion concentrate was emulsified with two volumes of water, and the seeds were soaked until they had absorbed all the liquid. The granulated formulation was sprinkled on the row of seeds before covering them with soil.

All the test seeds were planted at the following rates in flats containing 1 square foot of soil: cotton, 25 seeds (3 gm.); alfalfa, 100 seeds (300 mg.); and sugar-beets, 25 seeds (500 mg.). As the plants emerged they were sampled by collecting tissue from at least 10 plants per flat and were assayed for total radioactivity. The partition coefficients were assayed as well, in order to determine the amounts of active and degradation materials. The results of this experiment are shown in tables 1 and 2. Table 1 shows the results of seed treatment over a 2-month period after planting. In alfalfa, the initial concentration of toxicants in the leaves (calculated as parent compound) ranged from 19 to 143 p.p.m., and this had declined to 2 to 6 p.p.m. at the end of 2 months. In cotton the amounts were considerably higher, reflecting the smaller total seed surface in a given weight of seed (thus each seed carries a greater amount of toxicant), as compared with alfalfa. The amounts in the cotton leaves ranged from 45 to 340 p.p.m. initially, down to 18 to 48 p.p.m. after 2 months. In the case of sugar-beets the amounts ranged from 20 to 90 p.p.m. initially to 4 to 9 p.p.m. after 2 months.

Table 2.—Distribution of radioactivity (calculated as parent compound) in various leaf stages of plants 30 days after planting seeds coated with 50% activated charcoal at rates to give 4 ounces toxicant per 25 pounds of seed.

Material	P.P.M. Compound		
	In Cotyledons	In Unifoliate Leaves[a]	In True Leaves
Alfalfa			
Thiol-Systox	47.2	33.0	9.2
Thimet	55.2	37.0	12.2
Bayer 19639	50.8	38.4	9.8
Cotton			
Thiol-Systox	356.0		78.5
Thimet	63.4		13.8
Bayer 19639	120.6		8.5
Sugar Beets			
Thiol-Systox	62.4		27.8
Thimet	86.6		31.8
Bayer 19639	88.6		12.5

[a] In alfalfa, the first leaf following the appearance of the cotyledons is a unifoliate leaf in contrast to subsequent true leaves, which are trifoliate.

The data in table 2 are of particular interest because they show that the cotyledons of alfalfa, cotton, and sugar beets contain the greater part of the radioactivity and that this is not subsequently translocated to the true leaves in substantial amounts. Thus the toxicant exists in the plants in a considerable concentration gradient ranging from the highest value in the oldest leaves to the lowest in the youngest leaves.

The data obtained from this experiment indicate surprisingly little difference in the absorption of the toxicant following the three methods of seed treatment. Although the data are not conclusive, in general the emulsion concentrate appeared to be a little more rapidly absorbed than the others, while the granulated treatment persisted longest. This trend was born out in subsequent field experiments applied for insect control, and in residue studies.

The seed-treatment investigations were continued on a larger scale by planting alfalfa seed treated with 1 pound of Bayer 19639 per 25 pounds of seed (four times the rate

Table 1.—Total radioactivity (calculated as parent compound) in leaves of cotton, alfalfa, and sugar beet plants at indicated intervals after seed treatment (seeds treated at rate to give 4 ounces toxicant per 25 pounds of seed).

Treatment	P.P.M. Compound in Leaves at Intervals After Treatment														
	6 Days			10 Days			20 Days			39 Days[a]			59 Days		
	Thiol Systox	Bayer 19639	Thimet	Thiol Systox	Bayer 19639	Thimet	Thiol Systox	Bayer 19639	Thimet	Thiol Systox	Bayer 19639	Thimet	Thiol Systox	Bayer 19639	Thimet
Alfalfa															
Charcoal seed coat	43.2	63.8	143.0	13.5	22.4	35.2	9.2	8.5	12.2	6.5	9.3	5.1	3.2	2.6	2.8
Emulsion seed soak	18.8	21.5	23.7	9.2	42.4	54.8	6.0	12.3	15.1	4.8	11.9	8.3	1.8	3.3	6.0
Granules in seed row	21.4	83.2	48.4	10.6	39.5	15.9	9.3	18.3	10.3	15.6	11.4	15.5	6.6	4.9	4.6
Cotton															
Charcoal seed coat				213.0	43.0	82.5	78.5	9.8	13.8	76.2	37.6	31.9	40.1	18.6	23.8
Emulsion seed soak				339.0	288.7	206.0	63.7	18.2	33.8	102.9	51.8	68.2	37.5	31.2	37.7
Granules in seed row				186.0	125.3	87.3	33.8	58.5	39.4	168.6	123.2	137.1	26.0	47.8	32.1
Sugar beets															
Charcoal seed coat				21.0	52.5	56.8	27.8	12.4	31.8	22.6	6.8	9.9	5.7	4.3	3.8
Emulsion seed soak				27.5	45.2	89.5	34.0	22.2	79.6	21.7	11.2	45.9	7.1	4.5	9.0
Granules in seed row				20.4	59.5	31.4	23.2	51.4	36.2	17.0	32.4	17.2	8.5	4.4	7.0

[a] The $HCCl_3/H_2O$ partition coefficients of cotton leaves on this date were about 1 : 1, indicating that only half the apparent residue was active metabolites.

shown in tables 1 and 2 and that approximating field dosage), using 50% activated charcoal. This was applied as a slurry using 8% water per weight of seed with 2 ounces of methyl cellulose seed sticker per 25 pounds of seed. Three grams of Bayer 19639 were applied to 75 grams of seed, which was planted in a plot 5×40 feet. The distribution of radioactivity in the young plants is shown in table 3. It is evident that the initial concentration in the leaves was about 3 times that shown in table 1, but within 2 months the concentrations were nearly equal.

Here again the cotyledons contained from 3 to 10 times as much radioactivity as the trifoliate leaves, growing tips, stems, and roots. In all these tissues the activity decreased rapidly with time (see table 1 also). After 7 weeks the chloroform-partitioning material represented only about 20% of the total contamination.

As shown in table 4, the chloroform-partitioning fraction (active metabolites) decreased rapidly and at 8 weeks represented only trace amounts. At this time, cholinesterase assay with human plasma showed zero activity.

The plants described in tables 1 and 2 were infested with insects at various intervals after treatment to evaluate insecticidal efficiency. After 2 weeks the alfalfa seedlings treated with all of these compounds and all formulations were highly toxic to the spotted alfalfa aphid, *Therioaphis maculata* (Buckton), and killed from 90 to 100% of these aphids caged on the leaves for 3 days. At periodic intervals thereafter spotted alfalfa aphids were caged on the alfalfa plants. Approximately 1 month after planting, the treatments were just beginning to lose their effectiveness, indicating that a dosage of about 10 p.p.m. or perhaps slightly less is the breaking point in control of this insect.

After 2 weeks, the cotton plants were infested with the two-spotted spider mite, *Tetranychus telarius* (L.). No live mites were found after 3 days. This was continued throughout the experiment with no establishment of mites until sometime after the experiment was discontinued. The indication is that a relatively low amount of toxicant in the plant (less than 10 p.p.m.) will kill spider mites of this species.

After 20 days, beet leafhoppers, *Circulifer tenellus* (Baker) were caged on the sugar beet plants. One day later all the leafhoppers on the Thimet-treated plants were dead, from 0 to 20% of those on the Systox thiol isomer-treated plants were dead, and from 20 to 40% of those on the Bayer 19639-treated plants were dead. On this basis it must be assumed that Thimet is the most effective compound for control of the beet leafhopper.

In a later test, known amounts of P^{32} Thimet, Bayer 19639, and Systox thiol isomer were topically applied to cotton seed as the pure liquids. Sixteen days after planting, the total radioactivity in the entire plant and that remaining on the old seed husk were assayed. In this fashion it was possible to determine the approximate percentage of material which is absorbed into the plant, how much is left on the old seed husk, and how much is lost and cannot be accounted for. Results from the Thimet-treated seed showed that 8.8% was in the plant and 6.3% left on the seed; in the Bayer 19639 treatments there was 6.2% in the plant and 10.4% on the seed; and in the Systox thiol isomer treatments 2.8% was in the plant and 2.9% was left on the seed. It is probable that the smaller amount left on the seed in the Systox thiol isomer-treated plants is due to the greater water solubility of this compound (Metcalf *et al.* 1957), so that more of the compound is lost into the soil through the washing effect of irrigation.

EXPERIMENTS WITH ALFALFA.—Since the arrival of the spotted alfalfa aphid in California, the economics of growing alfalfa has changed drastically over much of the state's 1.2 million acres of this important crop. Damage by this pest to alfalfa has been described in previous publications by Tuttle & Butler (1954), Reynolds & Anderson (1955), and in greater detail by Parker *et al.* (1956). Prior to the arrival of this pest, it was seldom necessary

Table 4.—Residue of chloroform-soluble Bayer 19639 metabolites in alfalfa grown after seed treatment with P^{32}-Bayer 19639 at 1 pound per 25 pounds of seed, using 50% activated powder.

WEEKS AFTER TREATMENT	P^{32}-CHLOROFORM-SOLUBLE METABOLITES AS P.P.M. BAYER 19639 IN FRESH LEAVES
2	121.5
3	22.0
4	12.1
5	1.14
6	0.50
7	0.21
8	0.007[a]

[a] Cholinesterase assay of this sample showed 0.0 p.p.m.

Table 3.—Amounts of metabolites of Bayer 19639 in various parts of alfalfa plants grown in a 200-square-foot plot from seeds treated at a rate to give 1 pound of P^{32}-Bayer 19639 per 25 pounds of seed (formulated as 50% material on activated charcoal).

WEEKS AFTER TREATMENT	γ PER GRAM OF FRESH PLANT WEIGHT AS BAYER 19639											
	Total Plant		Growing Tip		Trifoliate Leaves		Cotyledons		Stem		Root	
	P^{32}[a]	$HCCl_3$[b]	P^{32}[a]	$HCCl_3$[b]	P^{32}[a]	$HCCl_3$[b]	P^{32}[a]	$HCCl_3$[b]	P^{32}[a]	$HCCl_3$[b]	P^{32}[a]	$HCCl_3$[b]
2	97	—	93	—	—	—	174	—	—	—	102	—
3	34	—	47	—	61	—	205	—	12	—	66	—
4	51	—	44	—	78	—	209	—	15	—	34	—
5	—	—	16	—	14	2.9	152	—	5	—	24	—
6	—	—	5	—	4	0.29	—	—	2	<0.25	5	1.25
7	—	—	—	—	2.8	<0.3	—	—	1.2	<0.28	5.8	0.78

[a] Total radioactivity in homogenate calculated as Bayer 19639.
[b] Radioactive portion partitioning into chloroform from water homogenate.

to use insecticides in order to establish a young field of alfalfa successfully. The spotted alfalfa aphid, however, can and frequently does decimate stands of alfalfa in the seedling stage of growth when populations are high in an area. Because of extreme susceptibility of seedling alfalfa to aphid attack and because such heavy aphid migrations are occasionally encountered that applications of insecticides such as parathion must be made every few days, successful protection of the small alfalfa plants has often been difficult. As shown in field experiments for control of this aphid, treatments with Bayer 19639 and Thimet at time of planting should help greatly in alleviation of this problem as these compounds are translocated into the new growth in toxic amounts for some time after germination.

Some 15 field experiments and a number of laboratory tests were made before successful dosages and practical application methods were determined. Obviously, it is not possible to include all the results obtained in this paper. Consequently, only a few of the more indicative experiments are presented in detail, and a brief discussion of other methods of application and of results with systemic insecticides tested but not shown must necessarily suffice.

Preliminary Field and Greenhouse Tests.—A preliminary field test was conducted in the Palo Verde Valley of Riverside County. Fifty per cent Thimet on activated charcoal was tumbled dry with Africa variety alfalfa seed at two rates and planted to give 1.0 and 0.5 pounds of Thimet per acre. During the planting process it soon became obvious that a dry mix was not satisfactory. Much of the Thimet clung to the sides of the treating and seeding device and at the higher application the seeding rate was drastically reduced. Thimet was also applied as a 2% granulated formulation and broadcast at a rate to give 1 pound of toxicant per acre. Demeton was broadcast as a 10% granulated formulation at a rate to give 0.5 pound of toxicant per acre. In addition, 0.25% solutions of Chipman R-6199[4] and demeton were made and seed was soaked in each for 3 hours. The soaked seed was dried overnight and planted the next morning. At this time the seed had already started to germinate. After the seed was removed the remaining solution was measured, and it was calculated that the seed had absorbed enough solution that 25 pounds of seed contained slightly more than 1 ounce of toxicant. Since the relative rate of absorption of toxicant to that of water is not known, this is no more than a rough estimate.

As soon as possible after broadcasting the treated and untreated seed, the granule formulations were broadcast and the plots were cultipacked immediately. The plots were irrigated the following morning. Plots in this test were 100 feet long by 36 feet wide and each treatment was replicated four times. Results are shown in table 5.

The results obtained in this test clearly show the potential value of treating at planting time with Thimet and demeton. The other compound, Chipman R-6199, gave poor results which may be because this material translocates to only a small extent from the point of application (Metcalf *et al.* 1957). Another reason may be because this compound has subsequently been found to be a relatively inefficient aphicide (Stern & Reynolds 1957). The demeton seed soak gave good results but due to the mechanical difficulties in soaking, drying, planting before germination, and getting irrigation water onto the field quickly eliminates this method of treatment from consideration for reasons of practicality. Both the Thimet and demeton granules were quite promising. The charcoal formulations of Thimet for reasons already mentioned were not efficient in a dry form as this will not cling to a hard, smooth seed such as alfalfa. The reversal in results with the two dosages of Thimet on charcoal may be directly attributed to this difficulty. Consequently, in subsequent tests, liquid-fixation or slurry techniques were used to stick powder formulations onto the alfalfa seed.

Following the above preliminary test for control of spotted alfalfa aphid on seedling alfalfa, a number of greenhouse experiments were conducted prior to undertaking further field work. In these studies it was found that from 2 to 4 ounces of toxicant per 25 pounds of alfalfa seed of demeton, thiol isomer of Systox, Thimet, and Bayer 19639 (which had not been tested previously) were effective for as much as 3 to 4 weeks. Subsequently, it was found that such low dosages were not effective in the field. In addition to providing several other valuable leads, the greenhouse studies did indicate that Thimet does not remain effective quite so long as the other compounds.

As stated previously, a large number of field tests were conducted before successful dosages and the most practical application methods were determined. These tests were highly informative in providing background information useful in the later, more conclusive field experiments. The results do not merit a detailed discussion, but a few of the more important features are of interest.

Demeton, Systox thiol isomer (Isosystox), and *O,O*-dimethyl *S*-2-(ethylmercapto)ethyl phosphorothioate (methyl demeton thiol isomer[5]) were tested thoroughly except for the last compound in comparison with Thimet and Bayer 19639. There were some indications that demeton, particularly the thiol isomer, translocated into the young plant in toxic amounts somewhat more quickly than the Thimet and Bayer 19639. This is substantiated by the work of Metcalf *et al.* (1957), who found that Systox thiol isomer is initially absorbed and translocated about 1.5 to 2 times as fast as Thimet and Bayer 19639. They suggest that this may be explainable by the comparative water solubilities of these compounds, in that Systox thiol isomer and, to a lesser extent, the thiono isomer, form oxidative metabolites which are more water soluble in the subcuticular layers of plant tissue around the region of application.

Demeton and Systox thiol isomer in these tests appeared to be about equal to Bayer 19639 and slightly superior to Thimet in control of the spotted alfalfa aphid. Methyl demeton thiol isomer appeared to be the least effective of all the compounds tested, although the data were not conclusive. In these preliminary tests, however, for effective aphid control in the field it appeared to be necessary to use amounts ranging from 8 to 16 ounces of material per 25 pounds of alfalfa seed. As the common agronomic practice in California is to plant from 20 to

[4] Produced by Imperial Chemical Industries, Ltd., England.
[5] Produced by the Chemagro Corporation.

Table 5.—Results of treating alfalfa seed and broadcasting granulated formulations of systemic insecticides to protect seedling alfalfa from attack by the spotted alfalfa aphid.

Treatment[a]		Oz. Toxicant per Acre[b]	Apterous Aphids per 100 Plants		Per Cent Infested Plants		
Material	Method		Dec. 22, 1955	Jan. 6, 1956	Dec. 5, 1955	Dec. 22, 1955	Jan. 6, 1956
Thimet	50% on Charcoal mixed dry with seed	16	99	435	1.8	29.5	63
Thimet	50% on Charcoal mixed dry with seed	8	47	188	1.5	19.0	34
Thimet	2% granule broadcast after broadcasting seed	16	16	86	0.9	4.5	14
Demeton	10% granule broadcast after broadcasting seed	8	7	123	1.2	3.0	22
Demeton	Seed soaked 3 hours in 0.25% solution	1	6	79	0.3	2.5	21
Chipman R-6199	Seed soaked 3 hours in 0.25% solution	1	131	730	5.3	40.5	79
Check	—	—	171	736	10.5	41.5	86

[a] Seed treated and planted November 9, 1955.
[b] Stand counts indicated no impairment of germination or plant stand in any of the treated areas.

25 pounds of seed per acre, for reasons of practicality, further experimentation on this problem with demeton and its various isomers was abandoned.

In addition to the systemic materials impregnated on activated charcoal for use in coating the seed, several other carriers were tested. It was not possible to impregnate Attaclay at a 50% level and yet retain a desirable formulation. A 25% formulation on Attaclay retained the desirable characteristics, but it was difficult to coat the seeds with the high rates necessary for good field control of the aphid.

Emulsifiable formulations were tested. Enough water was added to provide sufficient liquid to distribute the material uniformly over the seed. No impairment in germination was experienced at rates up to 8 ounces of toxicant per 25 pounds of seed, but the seed was too sticky to flow well in the seeding process. Stickiness could probably be eliminated after treating the seed by adding and thoroughly mixing some absorptive carrier such as Attaclay, or perhaps even a non-absorptive carrier. Due to the ease of using this latter method of treatment, further experimentation is perhaps justified.

The addition of a small amount of methyl cellulose[6] seed adhesive seemed to improve somewhat the adherence of the charcoal coating to the seed. This was added at the rate of from 2 to 4% by weight of the charcoal formulation or, preferably, 2 to 3% by weight of water added.

Experiment No. 1.—This is a combination of two tests, one in Kern County and the other in Tulare County, both areas in the San Joaquin Valley of California. Caliverde variety alfalfa seed was used in each test. These tests were started on May 4 and 17, 1956, respectively, a time of year when alfalfa seedlings grow very rapidly.

Fifty per cent Bayer 19639 on activated charcoal was applied as a seed coat at a rate to give 16 and 8 ounces of toxicant per 25 pounds of alfalfa seed. A charcoal formulation of 44% Thimet was applied at the rate of 14 and 7 ounces per 25 pounds of seed. The Thimet formulation contained 2% methyl cellulose and, in addition, methyl cellulose seed adhesive was added to both compounds at the rate of 1 ounce per 25 pounds of seed in the higher dosage and at the rate of 0.5 ounce in the lower dosage. Water in which the methyl cellulose had been dissolved was added to the seed at the rate of 1 quart per 25 pounds of seed at the higher dosage and 0.55 quart at the lower dosage. The charcoal material was added immediately to the wet seed and tumbled until the seed had a uniform coating. Two per cent granulated formulations of the Bayer 19639 and Thimet were broadcast at approximately 50 pounds per acre in order to obtain 1 pound of toxicant per acre. The seed and granules were broadcast onto the soil surface with a 10-foot mechanical seeder in the Kern County plots and with a hand-crank seeder in the Tulare County plots. In both cases the seed and granules were cultipacked almost immediately after seeding and the field was irrigated the next day. Each treatment was replicated three times in Kern County, and each individual replicate was 20 feet wide and 200 feet long. In Tulare County each replicate was 30 feet wide by 90 feet long and each treatment was replicated four times. The results of these tests are shown in table 6.

The results of these two experiments clearly show the value of the systemic treatments in reducing spotted alfalfa aphid populations on seedling plants. The results of these tests further substantiate the laboratory findings in that Bayer 19639 seed coating is somewhat superior to Thimet seed coating in maintaining the aphid population at a low level for a long period of time. There was little difference between 7 and 14 ounces of Thimet per 25 pounds of seed, with both dosages checking the aphid build-up for approximately 1 month when alfalfa grows rapidly as it was in these tests. The Bayer 19639 seed coatings were effective a little longer with the 16-ounce dosage being somewhat superior to the 8-ounce dosage. In the Kern County test both materials in the granulated formulation gave better results than the seed coating treatments although initially the two methods were about equal in effectiveness. In the Tulare County test the granulated formulation of Thimet was definitely inferior

[6] Material used in these tests was Colloidal Seed Coat, a methyl cellulose formulation manufactured by Colloidal Products Corporation.

Table 6.—Results of two experiments in which seed coatings and granulated formulations of Thimet and Bayer 19639 were applied to control the spotted alfalfa aphid on seedling alfalfa at the time of year when the young plants grow rapidly.

Material	Application Method	Amount Toxicant	Apterous Aphids per 100 Plants					Per Cent Infested Plants				
			Kern County Experiment[a]									
			May 23	*May 29*	*June 6*	*June 13*	*June 20*	*May 23*	*May 29*	*June 6*	*June 13*	*June 20*
Bayer 19639, 50% on charcoal	Seed coat	16 oz./25 lbs. seed	0	12	105	312	—	0	7	32	46	—
Bayer 19639, 50% on charcoal	Seed coat	8 oz./25 lbs. seed	5	34	150	805	—	3	13	30	62	—
Thimet, 44% on charcoal	Seed coat	14 oz./25 lbs. seed	4	75	478	905	—	4	23	59	62	—
Thimet, 44% on charcoal	Seed coat	7 oz./25 lbs. seed	11	137	265	570	—	7	34	45	57	—
Bayer 19639, 2% granule	Broadcast	14 oz./acre	2	16	23	144	364	2	10	15	29	50
Thimet, 2% granule	Broadcast	21 oz./acre	17	35	20	139	320	5	14	15	29	51
Check	—	—	65	320	463	1371	1655	36	75	86	74	90
			Tulare County Experiment[b]									
			June 7	*June 14*	*June 21*	*June 27*		*June 7*	*June 14*	*June 21*	*June 27*	
Bayer 19639, 50% on charcoal	Seed coat	16 oz./25 lbs. seed	0.5	2	22	60		0.5	1	8	21	
Bayer 19639, 50% on charcoal	Seed coat	8 oz./25 lbs. seed	0.0	1	63	148		0.0	1	16	36	
Thimet, 44% on charcoal	Seed coat	14 oz./25 lbs. seed	0.5	29	90	146		0.5	11	40	41	
Thimet, 44% on charcoal	Seed coat	7 oz./25 lbs. seed	1.5	11	77	118		1.5	7	31	37	
Bayer 19639, 2% granule	Broadcast	18 oz./acre	0.5	0	1	50		0.5	0	1	21	
Thimet, 2% granule	Broadcast	18 oz./acre	2.0	31	118	192		1.0	7	40	53	
Check	—	—	11	138	133	172		7.5	32	55	65	

[a] Seed treated and planted May 4, 1956.
[b] Seed treated and planted on May 17, 1956.

to those of Bayer 19639. As it is known that some early formulations of granulated Thimet were not stable, it is possible the Thimet content of the particular material used in this field had declined.

It should be noted that the alfalfa in these two tests grew rapidly. The alfalfa plants had attained a height of about 4 to 6 inches 6 weeks after planting, in contrast to the alfalfa in the following test, which attained this height after about 10 weeks.

Experiment No. 2.—This test was conducted in the Blythe area of eastern Riverside County. Africa variety alfalfa seed was treated and planted on October 26, 1956, at a time of year when the seedling alfalfa plants grow quite slowly.

Fifty per cent Bayer 19639 and 44% Thimet on activated charcoal were applied to the seed as a coating at the rate of 1.0 and 0.88 pound of toxicant per 25 pounds of seed, respectively. The seed was treated in the manner described in Experiment 1. Instead of tumbling the seed in order to make the coating adhere the seed was coated in a commercial feed-mixing mill which was sufficiently large to handle 100 pounds of seed at a time. The use of this equipment for applying the seed coat resulted in a uniform distribution and firm adherence of the charcoal material to the alfalfa seed. A 2% granulated formulation of Bayer 19639 was broadcast with the seeding equipment at a rate to give 1 pound of toxicant per acre. In addition, broadcast sprays of Bayer 19639 and Thimet were applied at the rates of 1.5 and 2.0 pounds per acre, respectively, to plots just after seeding. The sprays were applied at a pressure of 60 p.s.i. at the rate of approximately 7 gallons per acre. The sprayer was equipped with a 30-foot boom on which a low-volume hollow-cone nozzle was located every 18 inches. Immediately after the seed and various treatments were applied, all the plots were cultipacked. The field was irrigated the following morning. In this test each plot was 60 feet wide by 1240 feet long. Each treatment was replicated three times, so that the total area per treatment was about 5.1 acres. Results are shown in table 7.

In contrast to results obtained in Alfalfa Experiment No. 1, the treatments remained effective for a much longer period of time, the Thimet for about 8 weeks and the Bayer 19639 for about 10 weeks. There was little difference in results of the three methods of treatment at the rates used in this experiment. Past experience indicates that spray treatments as used in this experiment are not so efficient as the other methods of treatment in plant utilization of the systemic insecticide. Due to certain obvious advantages, however, the spray method at these and lower rates is being investigated further.

At the time of the last count the alfalfa had reached a

Table 7.—Results of a test in which seed coatings, granulated formulation, and broadcast sprays of Bayer 19639 and Thimet were applied at planting time[a] to control the spotted alfalfa aphid on seedling alfalfa. Blythe, Calif.

Material	Application Method	Amount Toxicant	Apterous Aphids per 100 Plants					Per Cent Infested Plants				
			Nov. 15	Nov. 27	Dec. 13	Dec. 20	Jan. 10	Nov. 15	Nov. 27	Dec. 13	Dec. 20	Jan. 10
Bayer 19639, 50% on charcoal	Seed coat	1.0 lb./25 lbs. seed	—	0	5	1	7	—	0	3	1	2
Thimet, 44% on charcoal	Seed coat	0.88 lb./25 lbs. seed	—	0	42	18	202	—	0	14	3	29
Bayer 19639, 2% granule	Broadcast	1.0 lb./acre	—	0.6	0	4	6	—	0.3	0	2	2
Thimet, emulsifiable concentrate	Broadcast spray	2.0 lbs./acre	—	1.3	11	2	217	—	1	5	2	33
Bayer 19639, emulsifiable concentrate	Broadcast spray	1.5 lbs./acre	—	0	0	0	8	—	0	0	0	6
Check	—	—	13.5	20.3	154	284	792	6	10	41	51	85

[a] Seed treated and planted October 26, 1956, at time of year when alfalfa grows slowly.

height of about 4 to 6 inches. As shown in the residue information obtained during this test, the materials dissipated completely shortly after the last count. Further counts could not be made as it became imperative to treat the check areas.

Discussion.—When aphid population levels on seedling alfalfa in California average one aphid per plant up to the time when there are five trifoliate leaves per plant, treatment is justified. Apparently Bayer 19639 or Thimet treatments will protect the small plant until and slightly after this less susceptible stage of growth is reached. The Bayer 19639 was somewhat superior to Thimet in spotted alfalfa aphid control but either material was satisfactory.

Interestingly enough, the results in Experiments 1 and 2 indicate that both materials are metabolized within the plants to nontoxic metabolites at differential rates apparently dependent to a large extent upon the rate of plant growth. The field results in both cases indicated that neither Thimet nor Bayer 19639 was still effective when the plants reached a height of about 6 inches, this being about 6 weeks in one case and 10 weeks in the other. This observation was substantiated by data obtained in the residue studies.

In plants from both the Bayer 19639- and Thimet-treated areas, there was a slight burn on the outer margins of many of the cotyledon leaves. In a few cases this was noticed on the unifoliate leaf to a slight extent, but never on the trifoliate leaves. In all cases the burn was somewhat more pronounced in the Thimet-treated plants. This may be associated with an accumulation at higher concentration levels of the systemic material at the termination on the vascular tissue. This burn was never enough to cause serious plant injury.

In none of the treatments could a significant reduction in germination be found. If plant stunting occurred, it was not serious enough to be noticeable. When compared with untreated seed, there was a tendency for the coated seed to flow through the planter at a slightly reduced rate.

EXPERIMENTS WITH COTTON.—A recent report by Parencia *et al.* (1957) discussed the use and potential value of Thimet and several other systemic compounds when used as treatments for cotton seed. They found that, of the materials tested, Thimet was the most promising and gave protection against attack by thrips (*Frankliniella* sp.), cotton aphid (*Aphis gossypii* Glov.), and possibly the serpentine leaf miner (*Liriomyza pusilla* (Meig.)). The materials used in their tests were effective initially, but not for the desired period of time against the cotton fleahopper (*Psallus seriatus* (Reut.)) and the boll weevil (*Anthonomus grandis* Boh.)

A number of field and laboratory experiments have been conducted in southern California since 1954. In most of these tests insect populations on the seedling cotton plants were characteristically low. It should be noted that in California, preventive or so-called "insurance" applications are not normally recommended. The exceptions are in nematode-infested areas where soil fumigation may be needed or when field conditions are conducive to seed-corn maggot (*Hylemya cilicrura* (Rond.)) or wireworm infestations, in which case seed coatings of an insecticide (plus a fungicide) often prove beneficial. Extensive experiments in evaluation of thrips control on seedling plants have shown no benefit in increasing yields under California conditions. Most insects which attack seedling cotton only seldom reach damaging populations. Such pests as flea beetles, darkling ground beetles, beet armyworms, fleahoppers, and others very rarely become abundant enough to necessitate treatment. Cotton aphids in occasional years reach pest status, particularly in the more northern cotton-growing areas of the state. Cutworms, principally the black cutworm, are the most serious of the seedling cotton pests in California, and a number of fields must be treated each year in order to prevent stand decimation. In some cases cutworm infestations can be predicted, as they are quite commonly abundant in cotton plantings that follow such crops as alfalfa.

Experiment No. 1.—This test was conducted in two fields in the Imperial Valley and in the greenhouse. Cotton seed was soaked in a number of systemic insecticide solutions and the results were compared with those obtained from seed treated with an activated charcoal seed coating. The seed was soaked for 24 hours in 1% solutions, dried, and planted immediately. Check seed was soaked in water and otherwise treated in a similar fashion. The plant stand obtained from the seed which had been soaked was sharply reduced in all cases, although the stand from seed soaked in technical schradan was not as badly reduced as that grown from seed soaked in the other materials which were fomulated with solvent and emulsifier. There was no reduction in stand grown from seed which had been coated with 50% Am. Cyanamid 12009 on activated charcoal at a rate to give 4 ounces of toxicant per 25 pounds of cotton seed. In contrast to alfalfa and certain other seeds, cotton seed is apparently more sensitive to soaking in systemic solutions.

Population levels of the various insects attacking seedling cotton were not high enough to evaluate in the field plantings. In the greenhouse the plants were artificially infested with cotton aphids and a spider mite, *Tetranychus cinnabarinus* (Boisd.). Demeton and Am. Cyanamid 12008 were effective in killing both species for some time after planting. Am. Cyanamid 12009 was effective on the spider mite, but gave poor results on the aphid, and schradan and diethyl 5-(3-methylpyrazolyl)phosphate[7] gave poor results on both species.

Experiment No. 2.—This test was conducted in the Imperial Valley on cotton variety Acala 4-42. Mechanically delinted cotton seed was treated with 50% Thimet on activated charcoal at a rate to give 1 pound of toxicant per 30 pounds of seed. The charcoal formulation was tumbled dry with the seed in a cement mixer until it adhered to the seed. The seed was planted on April 12, 1955. The treatment was replicated five times and each replicate or plot was 2 rows wide and ¼ mile long. Twenty-five plants were examined on May 5 and May 11. On May 24, when the cotton plants were 6 to 8 inches tall, 25 plants were examined for thrips, 75 cotyledons were examined for cotton aphids and spider mites, and 30 sweeps were made with an insect net for leafhoppers. Results are shown in table 8.

Stand counts showed that there were 1.4 plants per foot in the treated area and 1.6 plants per foot in the check area. It was felt that this was due primarily to the fact that the treated seed was planted at a reduced rate

[7] Also called Pyrazoxon; J. R. Geigy A. G.

Table 8.—Results of coating cotton seeds with Thimet for control of seedling cotton pests.[a] Imperial Valley, Calif.

Pest[b]	May 5[c]		May 11[c]		May 24[d]	
	Thimet	Check	Thimet	Check	Thimet	Check
Thrips (*Frankliniella sp.*)	1	151	3	58	52	66
Cotton aphid	1	few	few	few	0	6
Spider Mite (*Tetranychus atlanticus*)	0	few	—	—	3	21
Leafhopper (*Empoasca solana*)	—	—	—	—	104	330

[a] Seed treated at rate of 1 pound of Thimet per 30 pounds of seed; planted April 12, 1955.
[b] Some beet armyworms were observed in both the treated and the check areas, but there appeared to be less feeding damage in the treated area.
[c] Counts based on a total of 25 plants.
[d] Twenty-five plants were examined for thrips, 75 cotyledons were examined for aphids and spider mites, and 30 sweeps were made with an insect net for leafhoppers.

in comparison with untreated seed, rather than to reduced germination.

There was no significant difference in yield based on boll counts, although there was slightly more cotton in the check area at the time of the first picking.

Experiment No. 3.—This test was conducted on Acala variety 4-42 cotton in the Palo Verde Valley. Dry 50% Thimet on activated charcoal was added to mechanically delinted cotton seed and tumbled in a rotating drum for 15 minutes. The Thimet was used at rates to give 1.0 and 0.5 pounds of toxicant per 25 pounds of seed. The seeds were planted in plots which were 4 rows wide by ¼ mile long. Each treatment was replicated three times. Records were taken by examining 100 plants and 112 plants on May 26 and June 16, respectively. Plants were about 10 inches tall on June 11. Results are shown in table 9.

Experiment No. 4.—This test was conducted on Acala 4-42 variety cotton in the Palo Verde Valley. As the seed used in this test was acid-delinted, it was not possible to make the charcoal formulations adhere dry, so water was added and the materials were fixed with liquid onto the seed. Thimet 44D, an activated charcoal formulation containing 44% Thimet, was applied at rates to give 3.5 and 7 ounces of toxicant per 25 pounds of seed. Thimet was also applied as an emulsion at the rate of 4 ounces. Bayer 19639 was applied as an emulsion at the rate of 4 ounces per 25 pounds of seed, and a special soluble formulation containing 25% Bayer 19639 was used at the same rate of toxicant. In order to improve seed-coat adherence and, in the case of the emulsions, to reduce stickiness, methyl cellulose was added at the rate of 2 ounces per 25 pounds of seed. Water per 25 pounds of seed was used at the rates of 0.5 pint for the emulsions and 1.25 pints for the other treatments, except at the high rate of Thimet on charcoal, which required 1 quart. Each treatment was replicated four times and each plot or replicate was 4 rows wide and ¼ mile long. Results are shown in table 10.

Table 9.—Results of coating cotton seeds[a] with Thimet for control of seedling pests. Palo Verde Valley, Calif.

Pest	Pounds Thimet per 25 Pounds Seed[b]					
	May 26			June 16		
	1.0	0.5	0 (Check)	1.0	0.5	0 (Check)
Thrips (*Frankliniella sp.*)	0	0	67[c]	14	16	18[c]
Cotton aphid	1	0	8	3	15	18
Spider mite	0	0	0	1	0	3
Leaf miner (*Liriomyza sp.*)	—	—	—	1	5	15
Cabbage looper	—	—	—	4	6	4
Whitefly (*Trialeurodes abutilonea*)	—	—	—	0	2	10

[a] Seed treated and planted on May 5, 1955.
[b] Plants examined: 100 on May 26 and 112 on June 16.
[c] Additional 20 plants showed thrips injury.

Table 10.—Results of treating cotton seed[a] prior to planting with Thimet and Bayer 19639 for control of seedling pests. Palo Verde Valley, Calif.

Material	Oz. Toxicant/25 Lbs. Seed	Black Cutworm: Total Plants Cut per 400-Ft. Row April 26	Black Cutworm: Plants Cut Overnight 1-Mile Row April 27	Aphids[b]: Per 100 Plants May 8	Aphids[b]: Per 40 Leaves May 24	Thrips[b] per 40 Leaves May 24
Thimet, 44% on charcoal	7	7	10	8	10	187
Thimet, 44% on charcoal	3.5	17	26	11	51	187
Thimet, emulsion	4	2	—	10	13	223
Bayer 19639, emulsion	4	17	29	17	6	198
Bayer 19639, 25% soluble powder	4	18	—	3	5	248
Check	—	37	75	196	105	214

[a] Seed treated and planted April 11, 1956.
[b] Neither thrips nor aphids were present in the plots before these dates.

Experiment No. 5.—This test was conducted on Deltapine 15 variety cotton in the Imperial Valley. The seed was mechanically delinted, but the materials were applied onto dampened seed. One ounce of methyl cellulose, dissolved in water, was added per 25 pounds of seed and tumbled briefly; the 50% activated charcoal formulation was added immediately. The amounts of water used varied according to toxicant dosage, as follows: for 16 ounces of toxicant per 25 pounds of seed, 3 pints water; for 8 ounces of toxicant, 1.5 pints; and for 4 ounces of toxicant, 1 pint. Each treatment was replicated 4 times and each plot or replicate was 4 rows wide by ¼ mile long. The materials and dosages used and results obtained in this test are shown in table 11.

In a miscellaneous replicated test, Thimet 44D (44% Thimet) was applied at the rate of 2 pounds per 25 pounds of cotton seed. Four weeks after emergence a thrips count showed a reduction from 2.5 thrips per plant in the check plots to 0.25 thrips in the treated plots. A stand count taken in this field indicated a reduction of 29% in the number of plants in the treated areas. Here again this reduction appeared to be due primarily to a reduction in the planting rate with the treated seed rather than to impaired germination.

Discussion of Results.—In the field experiments on cotton, insect and spider mite infestations on the seedling plants were all relatively low. Seed treatments of Thimet and Bayer 19639 were effective on a surprisingly wide range of pests, however.

Thrips were effectively controlled by 1.0 and 0.5 pounds of Thimet per 25 pounds of seed for a period of between 4 and 5 weeks. Thrips populations were too low, however, to cause plant responses. Thrips were not present in plots where Bayer 19639 was applied.

Table 11.—Results of treating cotton seed[a] prior to planting with systemic insecticides for control of flea beetles on seedling cotton plants. Imperial Valley, Calif.

Material[b]	Ounces Toxicant per 25 Lbs. of Seed	On June 12: No. of Flea Beetle Feeding Scars on Cotyledons of 100 Plants	On June 12: Per Cent Reduction from Check
Thimet	16	213	76
	8	549	39
Bayer 19639	16	405	55
	8	426	52
Demeton	4	603	32
Thiol-isomer Systox	4	677	24
Check	—	893	—

[a] Seed treated and planted May 15, 1956.
[b] All materials formulated as 50% toxicant on activated charcoal.

The cotton aphid was controlled for approximately 5 to 6 weeks with Thimet at 1 pound per 25 pounds of seed, and the ½-pound dosage was effective for almost as long a period. Data were not conclusive, but Bayer 19639 appeared to be slightly superior to Thimet in control of aphids.

Populations of the southern garden leafhopper, *Empoasca solana* DeL., were reduced by about 66% 6 weeks after planting with 1 pound of Thimet per 25 pounds of seed. No data were obtained with other materials or dosages.

Populations of leafminers, *Liriomyza sp.*, and whiteflies, *Trialeurodes abutilonea* (Hald.), were quite low, but did not develop in plots grown from seed treated with Thimet at 1.0 and 0.5 pounds per 25 pounds of seed to the extent they did in the check plots.

Feeding damage by flea beetles was reduced 76% by Thimet at 1 pound per 25 pounds of seed and 39% at a ½-pound dosage. Bayer 19639 at 1.0 and 0.5 pound reduced the damage by 55 and 52%, respectively. Demeton and its thiol isomer at a 0.25-pound dosage gave reductions of 32 and 24%, respectively.

A few dead darkling ground beetles (*Blapstinus sp.*) were found in treated plots, but populations were too low to evaluate.

In one experiment a low population of the black cutworm, *Agrotis ypsilon* (Rott.), developed. Dosages of Thimet and Bayer 19639 were not above 7 ounces per 25 pounds of seed, but definite reductions in the number of cut plants were observed. In one 40-acre field about 10 acres were planted commercially with seed which had been treated with Thimet at the rate of 1 pound of toxicant per 25 pounds of seed. A medium infestation of cutworms appeared in this field and it was necessary to spray all the field except the portion which received the Thimet.

As mentioned by Parencia *et al.* (1957), cotyledons show burn on the outer margin at the 0.5 to 1.0 pound toxicant per 25 pounds of cotton seed, and burn is more pronounced at the higher rate. This was the case with both Thimet and Bayer 19639, although this phytotoxic effect was considerably less on plants grown from seeds treated with the latter material. The possible reason for this burn is discussed in the section of this paper dealing with alfalfa. When treated plants first appear they often have a slight yellowish color in comparison with plants which received no treatment. This is outgrown in less than 2 weeks. In addition, the cotyledons and the whole plant, when they first emerge from the soil, are slightly smaller than normal, but the first leaves are of normal size and any difference in the plant appearance and size is gone in about 2 to 3 weeks.

There can be no doubt that there were fewer plants in treated areas than in untreated areas. For the most part this apparently is because the seed with a heavy charcoal coating does not plant as rapidly as normal seed. Growers have found they must adjust their planters in order to plant the desired amount of seed per acre. It was shown that seed treated with systemic emulsions have a great reduction in germination, but the rate of germination was not affected markedly with the charcoal formulations under the conditions of these few experiments in southern California. Parencia *et al.* (1957) reported a partial loss of stand in treated plots when a heavy rain occurred the day after planting. Experience with sugar beet seed which had been coated with 1 pound of Thimet per 25 pounds of seed showed that inadequate moisture, or barely adequate moisture for untreated seed, caused a reduction in germination. In the case of the sugar beets, many seeds germinated at a later date when the field was irrigated. It is entirely possible that the activated charcoal may compete with the seed for the soil moisture. In California, where it is impossible to depend upon rainfall, practically all cotton fields are irrigated before planting. Thus the seed is planted in the mulch with adequate soil moisture. Few fields are irrigated immediately after planting. Under these cultural practices, possibly fewer difficulties with germination will be encountered than in areas of the cotton belt which depend upon rainfall for moisture. Prolonged, cool periods after planting, however, may cause adverse effects on germination.

EXPERIMENTS WITH SUGAR BEETS.—In a number of preliminary experimental tests in the field and in the laboratory, coatings and granulated formulations of systemic materials were applied at planting time on sugar beet seed. In the southern California area the two primary pests on seedling sugar beets are the beet armyworm, *Laphygma exigua* (Hbn.), and the beet leafhopper, *Circulifer tenellus* (Baker). The latter species is, of course, the vector of the highly destructive curly-top virus.

Thimet and Bayer 19639 were used in all these tests, and demeton was included in one. Fifty per cent demeton formulated on activated charcoal, when used as a seed coating at the rate of 0.25 pound of toxicant per 25 pounds of seed, was not promising for control of the beet leafhopper. When Thimet and Bayer 19639 were tested as seed coatings at the rate of 1 pound of toxicant per 25 pounds of seed, Thimet was considerably the more effective of the two. This agrees with the previously discussed data obtained in the laboratory in studies with radiotracers. It appeared that Thimet used at the above rate will protect the seedling sugar beet plants for a period of

from 2 to 3 weeks. This agrees quite closely with data reported by Hills *et al.* (1956). At the end of this period of time the sugar beet plants are in the 4- to 6-leaf stage, at which time the variety of beets grown in the Imperial Valley has attained a certain amount of tolerance to the curly-top virus. The possible effect of the protection against beet leafhopper attack and the relation to incidence of curly top is still being studied.

Protection of the seedling beet plant from beet armyworm attack was quite variable. In most experiments the reduction in armyworms was insignificant, but in one field the reduction in population with both Bayer 19639 and Thimet was about 50%. In all fields the dosage was the same, 1 pound of toxicant per 25 pounds of seed. The difference is believed to be due largely to variable irrigation practices. In the experiment where the reduction occurred, the grower irrigated the plants with overhead sprinklers. That this method provided insufficient moisture for good germination for the charcoal-coated seed in this particular instance is evidenced by the fact that there was about a 50% reduction in plant stand in the treated plots. The possible reason for this reduction was discussed above, but it is interesting to note that when the grower furrow-irrigated at a later date, many more treated seeds germinated.

The plants which grew in the treated areas had typical systemic burn on the cotyledons, indicating that considerable toxicant had accumulated in these portions of the plant. In contrast, in the fields in which no reduction in armyworms occurred, the cotyledons did not show this burn, which probably indicates less toxicant in this portion of the plant. The seed in these latter fields was irrigated by furrow irrigation. The weather in Imperial Valley in September is still extremely hot and dry, and the common agronomic practice is to practically keep water standing in the furrows until the seed has germinated and the small plants become established. It is reasoned that this excessive irrigation water causes a loss of toxicant through washing, dissolving, and carrying a certain percentage of the insecticide beyond reach of the young plant.

When 2% granulated formulations of Bayer 19639 and Thimet were drilled into the soil with the seed at a rate to give 1 pound of toxicant per acre, the resulting control of beet leafhopper was about the same as when these materials were coated on the seed at the rate of 1 pound of toxicant per 25 pounds of seed. When the fact is considered that for sugar production, beets are normally planted at the rate of from 5 to 8 pounds of seed per acre, it is obvious that the seed coatings conserve considerable insecticide.

Where adequate moisture was present during the warm time of year of these tests, no reduction in stand occurred when 1 pound of toxicant per 25 pounds of seed was used. When this rate was doubled, it did not appear that the stand was hurt in any way, but due to a high salt content in this field, results were not conclusive.

In initial tests, coatings were applied to the seed as slurries. In later tests the charcoal formulations were applied dry, and due to the corky, rough surface of the decorticated sugar beet seed it was not necessary to use moisture if the seed was tumbled for a period of 10 to 15 minutes.

EXPERIMENTS WITH SORGHUM AND SOY BEANS.—Thimet and Bayer 19639 were applied as seed coatings and granules to sorghum (Ryer 15 variety) and as seed coatings to soy beans (Lee variety) for control of the lesser cornstalk borer, *Elasmopalpus lignosellus* (Zell.). Seed coatings were applied at about 1 pound of toxicant per 25 pounds of seed on both crops and the materials were slurried onto the seed. Two per cent granules of both materials were drilled in with the sorghum seed at a rate to give nearly 1 pound of toxicant per acre. In the case of the soy beans, germination was almost completely inhibited. In the sorghum plots Bayer 19639 granules reduced the plant stand by 20% and the seed coating by 61%. The Thimet granules reduced the stand by 38% and the seed coating by 72%. Thimet was a little more severe than Bayer 19639 in reducing plant stand of both crops, and in the case of sorghum the stand reduction was considerably less with the granules than with the seed coatings. There seemed to be little or no reduction of lesser cornstalk borer larvae in any of the treatments.

RESIDUE STUDIES ON ALFALFA.—Thimet and Bayer 19639 residues on alfalfa were determined by the electrometric cholinesterase procedure of Hensel *et al.* (1954) briefly described as follows. The alfalfa was quartered down to 100 gm. and ground in a Waring Blender with 300 ml. distilled water for 3 minutes. The homogenate was filtered through 3 layers of cheesecloth, and the residue remaining on the cheesecloth was squeezed dry. The volume of filtrate consistently totaled 275–280 ml. The pH of the filtrate was adjusted to 7.0 by adding a few drops of aqueous potassium hydroxide, and the volume was adjusted to 300 ml. A 35-ml. aliquot was placed in a 50-ml. volumetric flask; 5 ml. of human blood plasma was added, and the total volume was made up to 50 ml. with neutral water. (In these calculations it was assumed that the weight of alfalfa in 35 ml. of filtrate is simply (35 /300) (100 gm.) = 11.7 gm.) The flask was kept at 37.5° C. for 70 minutes. A 1-ml. aliquot was then placed in a test tube along with 1 ml. of Veronal buffer (pH 8.00) and kept at 37.5° C. for 10 minutes; the pH was then measured. A 0.2 ml. solution of 0.66 M acetylcholine bromide was added to the test tube, the mixture was kept at 37.5° C. for 2 hours, and the final pH measured. The per cent inhibition was calculated in the usual manner.

To increase the sensitivity of the method, especially when the residues had fallen down to a low level (at 62 and 76 days), the procedure was modified by homogenizing the alfalfa in 300 ml. of methyl alcohol. The methyl alcohol-alfalfa mixture was filtered, the filtrate concentrated to dryness under vacuum, the residue taken up in water and carried through the usual procedure. By this modification the sample weight taken for analysis is increased 8.5-fold, and the lower limit of detectability is therefore increased by the same amount. In view of the greater solubility of Thimet, Bayer 19639, and their metabolites in methyl alcohol, a water-miscible solvent, it was expected that these materials would be completely extracted from plants by homogenization in methyl alcohol. This was verified in fortification experiments in which 100% recoveries of inhibitors were consistently obtained. Fortification experiments using water gave recoveries ranging from 85 to 100%.

It has been shown by Metcalf *et al.* (1957) that both

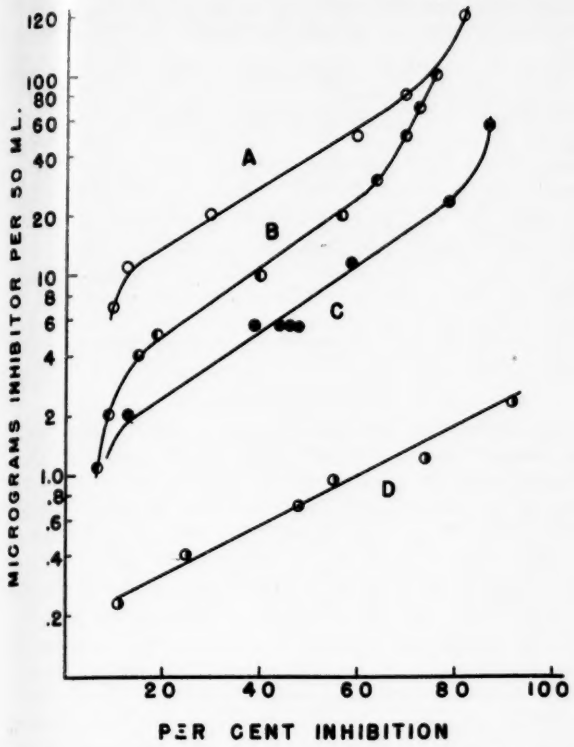

FIG. 1.—Standard curves showing relative inhibition of human plasma cholinesterase by Thimet and Bayer 19639 metabolites as determined by electrometric technique: (A) Systox thiol-isomer sulfoxide; (B) Systox thiol-isomer sulfone; (C) Thimet oxygen analogue sulfoxide; and (D) Thimet oxygen analogue sulfone.

Thimet and Bayer 19639 are metabolized in plants in a series of consecutive reactions to known oxidation products. It was demonstrated that the eventual major metabolites from Thimet are the oxygen analogue sulfoxide and sulfone *O,O*-diethyl *S*-(ethyl-2-sulfinyl)methyl phosphorothiolate and *O,O*-diethyl *S*-(ethyl-2-sulfonyl)methyl phosphorothiolate, respectively. Analogously, the eventual major metabolites from Bayer 19639 were found to be Systox thiol-isomer sulfoxide and sulfone, *O,O*-diethyl *S*-(ethyl-2-sulfinyl)ethyl phosphorothiolate and *O,O*-diethyl-*S* (ethyl-2-sulfonyl)ethyl phosphorothiolate, respectively.

In view of these previous findings, the residues of Thimet and of Bayer 19639 were calculated in terms of both sulfoxides and sulfones of the phosphorothiolates. Figure 1 shows the standard curves obtained with all four inhibitors. Plotted are micrograms inhibitor per 50 ml. against percentage of inhibition. These curves were obtained by adding calculated amounts of inhibitor to the alfalfa-water homogenate and proceeding according to the method of Hensel *et al.* (1954).

The residues of Thimet and Bayer 19639 found in seed-treated alfalfa at various time intervals after planting were calculated as p.p.m. of the respective sulfoxide and sulfone phosphorothiolates. Residues from alfalfa test 2 are presented in table 12.

Residue analyses were also made from alfalfa test 1. In this experiment samples were taken only once—41 days after planting. The modified procedure for increasing the sensitivity of analysis was not followed in this case. The results of analysis are shown in table 13.

From the data shown in tables 12 and 13, it does not appear that there will be a residue problem at the time of the first cutting or pasturing of alfalfa following treatment of systemic insecticides at planting time at the rates used in these experiments. The toxic metabolites of both Thimet and Bayer 19639 are apparently changed to nontoxic products at rates varying somewhat with the rate of plant growth. Thus when alfalfa grows slowly, toxic residues persist for about 8 weeks, and when the plants grow rapidly, residues persist for about 6 weeks. In either case, residues had dissipated when the plants attained a height of about 6 inches.

SUMMARY.—Systemic insecticides applied to seed of cotton, alfalfa, and sugar beets at time of planting were effective in protecting the seedling plants from attack by a surprisingly wide range of insects.

Radiotracer studies were conducted with P^{32} Bayer 19639, Thimet, and Systox thiol isomer. It was found

Table 12.—Residues of Thimet and Bayer 19639 on seedling alfalfa following treatment at time of planting.[a]

Material	Application Method	Amount of Toxicant	P.P.M. at Days Indicated After Planting				P.P.M. at Days Indicated After Planting			
			32	48	62	76	32	48	62	76
			Systox-Thiol Isomer Sulfoxide				*Systox-Thiol Isomer Sulfone*			
Bayer 19639, 50% on charcoal	Seed coat	1.0 lb./25 lbs. seed	4.5	1.1	<0.1	<0.1	1.9	0.39	<0.03	<0.03
Bayer 19639, 2% granule	Broadcast	1.0 lb./acre	4.7	1.1	<0.1	<0.1	2.1	0.39	<0.03	<0.03
Bayer 19639, emulsion spray	Broadcast	2.0 lbs./acre	2.3	1.2	<0.1	<0.1	0.90	0.43	<0.03	<0.03
			Thimet Oxygen Analogue Sulfoxide				*Thimet Oxygen Analogue Sulfone*			
Thimet, 44% on charcoal	Seed coat	0.88 lbs./25 lbs. seed	3.0	0.21	<0.02	<0.02	0.36	0.03	<0.003	<0.003
Thimet, emulsion spray	Broadcast	2.0 lbs./acre	3.06	0.48	0.03	<0.02	0.43	0.05	<0.003	<0.003

[a] Seed treated and planted October 26, 1956, at time of year when alfalfa grows slowly.

Table 13.—Residues of Thimet and Bayer 19639 on seedling alfalfa following treatment at time of planting.[a]

Material	Application Method	Amount of Toxicant	P.P.M. 41 Days After Planting	
			Systox-Thiol Isomer Sulfoxide	
Bayer 19639, 50% on charcoal	Seed coat	16 oz./25 lbs. seed	<1.0	
Bayer 19639, 50% on charcoal	Seed coat	8 oz./25 lbs. seed	<1.0	
Bayer 19639, 2% granule	Broadcast	18 oz./acre	<1.0	
			Thimet Oxygen Analogue Sulfoxide	*Thimet Oxygen Analogue Sulfone*
Thimet, 44% on charcoal	Seed coat	14 oz./25 lbs. seed	<0.1	<0.02
Thimet, 44% on charcoal	Seed coat	7 oz./25 lbs. seed	<0.1	<0.02
Thimet, 2% granule	Broadcast	18 oz./acre	0.14	0.02

[a] Seed treated and planted May 17, 1956, at time of year when alfalfa grows rapidly.

that following seed treatment the cotyledons of alfalfa, sugar beets, and cotton contained the highest concentrations of toxicant and that this is not translocated in substantial amounts to other plant parts. The toxicant exists in the plants in a concentration gradient ranging from the highest value in the oldest leaves to the lowest in the youngest leaves. There were little differences in absorption of the toxicant following treatment of the seed with an activated charcoal seed coating, with an emulsion, or by placing granulated formulations in the seed row. It was found that 10 p.p.m. or a little less was toxic to the spotted alfalfa aphid, *Therioaphis maculata* (Buckton), less than 10 p.p.m. was toxic to the two-spotted spider mite, *Tetranychus telarius* (L.), and that Thimet was more effective than Bayer 19639 and Systox thiol isomer in killing the beet leafhopper, *Circulifer tenellus* (Baker).

In order to protect the seedling alfalfa plants from the extreme damage caused by the spotted alfalfa aphid, a number of systemic insecticides were tested as seed coatings, and as broadcast applications of granulated formulations and emulsion sprays. Demeton, Systox thiol isomer, and Bayer 19639 were about equal in effectiveness and just slightly superior to Thimet. Methyl demeton thiol isomer and Chipman R-6199 gave relatively poor results. When alfalfa seed was coated with Thimet and Bayer 19639 on activated charcoal at a rate of 8 ounces of toxicant per 25 pounds of seed, good aphid control was maintained for a period of about 4 to 5 and 5 to 6 weeks, respectively, when the seed was planted at a time of year when alfalfa grows rapidly. A rate of 16 ounces per 25 pounds of seed improved results only slightly. When seed was planted at a time of year when alfalfa grows quite slowly, aphid control persisted about another 4 weeks. Effectiveness was lost in either case when the plants attained a height of about 6 inches. Granulated Thimet and Bayer 19639, when broadcast at a rate to give 1 pound of toxicant per acre, were a little more effective than seed coatings after a period of time, but about equal initially. Emulsion sprays of Thimet and Bayer 19639 applied with a sprayer over the seeded field prior to cultipacking or harrowing in the seed gave excellent results at the rate of 2 pounds per acre.

Thimet and Bayer 19639 were tested as seed treatments on cotton, and to a lesser extent demeton, Systox thiol isomer, schradan, and Pyrazoxon were also tried. The latter three compounds were relatively ineffective in greenhouse tests. Although initial work showed Am. Cyanamid 12008 and 12009 to be promising, they were abandoned as Thimet and Bayer 19639 gave somewhat superior results. Thimet seed coatings at the rate of 1.0 pounds of toxicant per 25 pounds of seed controlled thrips (*Frankliniella sp.*) for about 4 to 5 weeks, cotton aphid, *Aphis gossypii* Glov., for about 5 to 6 weeks, reduced populations of southern garden leafhopper, *Empoasca solana* DeL., by 66% 6 weeks after planting, and flea beetle damage by 76%. Thimet at 0.5 pound was nearly as effective as at 1.0 pound except on flea beetles. Thimet also shows promise in controlling whiteflies (*Trialeurodes abutilonea* (Hald.)), leafminers (*Liriomyza sp.*), darkling ground beetles (*Blapstinus sp.*) and black cutworms (*Agrotis ypsilon* (Rott.)). Bayer 19639 was not tested on as wide a range of cotton pests, but at the same rate as Thimet was equally promising on cutworms, slightly superior on aphids, and less effective on flea beetles. Demeton and Systox thiol isomer at 0.25 pound of toxicant per 25 pounds of seed were not satisfactory for control of flea beetles. Although results from greenhouse tests show these materials to be highly promising on spider mite control, these pests did not develop in numbers in the field that results could be evaluated.

Experiments by treating sugar beet seed showed Thimet at 1.0 pound per 25 pounds of seed to be effective in controlling the beet leafhopper for about 2 to 3 weeks. Reductions in beet armyworm, *Laphygma exigua* (Hbn.), populations were variable, and not considered promising. Bayer 19639 at this rate and Systox at 0.25 pound were inferior to Thimet for control of beet leafhopper.

Many cotyledons of newly emerged cotton, alfalfa, and sugar beet plants showed some burn on the outer margins with both Thimet and Bayer 19639 treatments. It was more pronounced at the higher rates, but did not appear on the leaves. Of the two materials, the burn was more pronounced in the case of Thimet. Seedling cotton plants show some reduction in size but this difference disappears in about 2 to 3 weeks. Treatment of cotton seed with systemic emulsions affected germination adversely, but activated charcoal formulations did not affect germination to any marked extent under conditions of these tests. The heavy charcoal seed coatings did reduce the rate of seed flow through the planter, however. When soil moisture was adequate, sugar beet and alfalfa stands were not reduced by treatment.

Germination of soy bean seed, when coated with

Thimet and Bayer 19639 at 1.0 pound toxicant per 25 pounds of seed, was almost completely destroyed. Sorghum seed germination was greatly impaired at this rate, as well, although Bayer 19639 seed coatings affected the seed less than Thimet and granulated formulations were safer than seed coatings.

Residue studies on alfalfa following systemic insecticide treatment at planting time showed that there was no residue problem by the time of first cutting or pasturing. Toxic residues had dissipated in about 6 to 8 weeks with either Thimet or Bayer 19639 regardless of method of treatment. Regardless of the rate of plant growth, residues were gone when the small plants reached a height of about 6 inches.

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Corn Earworm Control in Grain Sorghum[1]

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One of the major insect pests attacking grain sorghum in Kansas is the corn earworm, *Heliothis zea* (Boddie). Although larvae feed on leaves to some extent, the economic losses occur from feeding in the heads. Early instars hollow out the kernels while third instars hollow out larger kernels and consume varying proportions of the kernels. Third to sixth instars completely consume tremendous numbers of the kernels. This feeding causes: (1) loss in germination and (2) loss in weight or yield. As high as 100% of the sorghum heads have been infested in some fields, with some heads harboring as many as 16 larvae.

Burkhardt & Breithaupt(1955) obtained excellent control with Phosdrin at 0.5 pound per acre, resulting in 96 and 100% kills after 24 and 96 hours, respectively. DDT at 2.75 pounds per acre gave the second highest percentage of control. Aldrin and DDT-TEPP combination gave control according to Dahms *et al.* (1955).

Aerial application of 0.5 pound Phosdrin per acre gave 95 and 100% control after 1 and 4 days, respectively, in tests by De Pew (1957) in southwestern Kansas during 1956. DDT at 2 pounds per acre, also applied aerially at the rate of 3 gallons of total emulsion per acre, gave less effective control.

MATERIALS AND PROCEDURES.—A series of tests were

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conducted near Manhattan during August and September, 1956. DDT, DDT-toxaphene, endrin, heptachlor, malathion, methoxychlor, parathion, Phosdrin, and Thimet were evaluated at various rates with different carriers, gallonages per acre, and methods of application. Only the emulsifiable concentrate formulations of the above insecticides were used. All materials were applied to the grain sorghum in the head stage of development. Four experiments designated Test A through D are discussed herein.

Test A.—Insecticides in this test were applied August 28, 1956, with a 3-gallon, compressed-air hand sprayer, using 30 pounds pressure and applying 20 gallons of emulsion per acre. Each plot was replicated. Previous to treatment 95% of the heads were infested with 1 to 5 larvae per infested head. The results of this test were based on careful examination of 20 random heads from each plot, 1 and 3 days after treatment.

Test B.—Insecticides were applied September 1, 1956, with a Brodjet tractor-mounted sprayer, using 40 pounds pressure and applying 14 gallons of total emulsion per acre. Each plot consisted of 18 rows the length of the field, or approximately 1 acre in area. Approximately 90% of the heads was infested with 1 to 5 larvae per head. Readings were taken on 40 random heads in each plot, 1 and 4 days after treatment.

Test C.—All insecticides were applied September 10, by aerial application. Heptachlor and parathion were applied in water as well as in diesel fuel at the rate of 4 gallons and 1 gallon total emulsion per acre, respectively. Because of a limited supply of Phosdrin, it was applied in diesel fuel only at the rate of 0.375 pound of toxicant per acre. Materials were applied at a flight height of about 24 inches above the plants. Swath was limited to the width of the boom. Each plot, including an untreated plot, consisted of 5 acres. At the time of treatments, 83% of the heads was infested with 1 to 5 larvae per head. Forty heads were examined in each plot, 1, 4, and 7 days following treatment.

Test D.—Only Phosdrin at two rates, along with an untreated check, were studied. Applications were made September 11 with a 3-gallon compressed-air sprayer operating at 30 pounds pressure and applying 20 gallons of emulsion per acre. Ninety per cent of the heads was infested, ranging from 1 to 6 larvae per head

Effectiveness of treatments was based on the number of larvae in treated heads compared with the number in untreated heads, and was recorded as percentage reduction of larvae.

RESULTS AND DISCUSSION.—Results are summarized in table 1.

Test A. Table 1 shows that Phosdrin at 0.5 pound per acre exhibited 97% control in 24 hours, followed by 2.5 pounds of DDT with 94%. All other treatments resulted in less than 90% reduction 1 day after treatment. Both Phosdrin and DDT gave 100% reduction after 3 days. Endrin at 0.2 and 0.4 pound per acre resulted in a slower kill but averaged 95% 3 days after treatment.

Test B.—Table 1 also shows comparative percentages of control 1 and 3 days after treatment. Phosdrin, at 0.375 pound per acre, exhibited the highest kill, 87%, in 24 hours. DDT at 2 pounds per acre rated second best. DDT and Phosdrin gave comparable results after 3 days, resulting in 92 and 95% kills, respectively. All other treatments gave inadequate control.

Table 1.—Control of corn earworm in sorghum heads by insecticides applied with different types of equipment. Manhattan, Kansas. 1956.

Treatment	Pounds of Toxicant per Acre	Per Cent Reduction of Larvae After Treatment on Days Indicated			
		1	3	4	7
A. Compressed-air hand sprayer, August 28					
DDT	2.5	94	100		
	1.5	87	100		
Endrin	0.4	81	95		
	0.2	65	95		
Heptachlor	1.5	65	63		
DDT-toxaphene	1.2	77	74		
Phosdrin	0.5	97	100		
Parathion	0.5	87	89		
Thimet	1.0	61	84		
B. Brodjet-type tractor-mounted sprayer, September 1					
DDT	2	81	95		
Methoxychlor	1.5	27	41		
	2.0	48	17		
Malathion	1.0	75	56		
	2.0	76	75		
Phosdrin	0.375	87	92		
C. Aerial Applications, September 10					
Heptachlor (water)	1.5	40		82	67
Heptachlor (diesel oil)	1.5	43		86	67
Parathion (water)	0.75	39		83	67
Parathion (diesel oil)	0.75	53		82	58
Phosdrin (diesel oil)	0.375	61		92	58
D. Compressed-air sprayer, September 11					
Phosdrin	0.25	69		82	91
	0.5	87		91	100

Test C.—The results of aerial applications are also summarized in table 1. Phosdrin at 0.375 pound per acre resulted in the highest kill in both the 1 and 4 day post-treatment counts, 61 and 92%, respectively. Both heptachlor and parathion gave quicker kill in diesel fuel at 1 gallon emulsion per acre than in water at 4 gallons emulsion per acre. However, the differences became less extensive 4 and 7 days after treatment. Parathion at 0.75 pound was inferior to Phosdrin at 0.375 pound per acre.

Test D.—Although 0.25 pound of Phosdrin per acre resulted in rather low reduction, 69% 1 day after treatment, 0.5 pound gave 87%. Both rates provided increased reduction of larvae 4 and 7 days after treatment as shown in table 1, D.

Whenever Phosdrin and DDT were included in the same tests, they rated first and second, respectively, in performance. However, DDT at present involves a residue problem and cannot be recommended. Both endrin and heptachlor gave relatively low control during the first 24 hours. As much as 2 pounds of methoxychlor per acre was ineffective. Of the organic phosphorous insecticides, Phosdrin at 0.375 pound per acre resulted in better control than parathion at 0.75 pound per acre. Malathion at 2 pounds per acre resulted in moderate control.

Aerial applications gave slower kills than ground applications. Rapid kill is important because of the voracious

feeding of the corn earworms. An additional day of feeding will incur a considerable increase in loss.

From the data presented in table 1 as well as from unreported data, it is evident that applications made with a Brodjet-type sprayer were inferior to applications made with a boom-type sprayer.

SUMMARY.—The corn earworm is one of the major insect pests of grain sorghum in Kansas. Its feeding in the heads results in a reduction in germination and in grain yield. As high as 100% of the heads were infested in some fields, with some heads harboring as many as 16 larvae.

Chemical control tests were conducted near Manhattan during August and September, 1956. DDT, DDT-toxaphene, endrin, heptachlor, malathion, methoxychlor, parathion, Phosdrin, and Thimet were evaluated. Phosdrin at 0.375 and 0.5 pound per acre consistently gave the quickest and highest kill. At 0.25 pound per acre results were less satisfactory. DDT resulted in good control at 1.5 to 2.5 pounds per acre but cannot be recommended at present, except for seed production, because of the residue problem. Endrin, heptachlor, parathion, and malathion exhibited fair control.

Aerial application gave somewhat slower kill than ground applications, and using a Brodjet-type ground sprayer resulted in poorer control than using a boom-type ground sprayer.

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Status of the Cooperative Gypsy Moth Program September 30, 1956[1]

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The most serious outbreak of the gypsy moth, *Porthetria dispar* (L.), on record occurred in the summer of 1953 when nearly 1½ million acres of New England woodlands were defoliated by this pest. Perhaps of greater significance than the magnitude of the outbreak was the fact that much of the defoliation occurred for the first time in areas west of the principal Berkshire and Green Mountain elevations. There was apprehension at the time that existing high populations in this area, located only a few miles from the western limits of general infestation, would result in considerable spread of the moth into previously uninfested territory. These fears were amply justified as demonstrated by the results of detection surveys conducted in succeeding years.

SPREAD OF INFESTATION AND SUBSEQUENT ACTION.—A large-scale trapping survey is conducted each summer, principally for the purpose of delimiting the western periphery of general infestation and for locating isolated spots of infestation which may have become established in outside territory. In both the 1954 and 1955 programs male gypsy moths were captured to the western edge of the general area under survey. These surveys, and the finding of egg masses at various sites where moths had been captured, showed conclusively that spread of the pest had occurred on a scale unequaled since its introduction at Medford, Mass., some 86 years ago. The magnitude of spread is evidenced by recent revisions in State and Federal gypsy moth quarantines, in which approximately 10 million acres of additional territory have been brought under regulation.

After results of surveys in 1955 were published, much concern was expressed by various Agricultural and Conservation groups across the country relative to the potential threat to our forest resources should the gypsy moth continue to spread into the highly susceptible woodlands of the south and mid-west. Developing interest in this problem led last March to a State-Federal conference to review the current situation and to consolidate plans for future cooperative action. The discussion developed as an immediate goal the elimination of known infestations in Michigan, New Jersey and Pennsylvania, and the spraying of a protective border in southern New York. Tentative long-range plans called for a further expansion of the program in 1957 and succeeding years, looking to the eventual eradication of the pest.

PAST ACCOMPLISHMENTS.—A review of past experience in gypsy moth suppression has shown that the insect was successfully eliminated from both large and small areas in which infestation had become established in outlying areas. An outstanding example of such an accomplishment is the extermination work completed in New Jersey in 1935. Here an infested area comprising some 1,450 square miles was cleaned up even though the tools and procedures used have long since been outmoded.

In 1932 gypsy moth infestation was found to be established throughout extensive territory in and around Scranton, Pennsylvania. Subsequent surveys revealed infestation to be distributed over approximately 1,000 square miles of territory. After DDT became available for general use, this area of infestation was cleaned up after four seasons of progressive aerial spray operations, such treatments being completed in 1949.

The most recent example of successful, large-scale operations has occurred in Michigan where in 1954 infestation of undetermined origin was found in the city of Lansing. A condition of general infestation was found to exist throughout approximately 100 square miles of area. Additional spot infestations were found throughout an even greater expanse of peripheral territory as a result of continuing surveys. More than 221,000 acres have been aerially sprayed in three seasons of control operations. In

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a recently completed trapping program only a single male gypsy moth from each of two traps was recovered from the 4,900 traps employed in a survey covering nearly 1½ million acres. This seems to provide substantial evidence that the program in Michigan has reached the point where early eradication can be achieved through a continuation of intensive survey and mopup spray operations.

BEGINNING OF AN ACCELERATED CONTROL AND SURVEY PROGRAM.—With the available evidence that gypsy moth infestation can be eliminated from any given area, funds were sought in the spring of 1956 with which to initiate an over-all eradication effort. This program has an initial objective of eradicating the pest from territory newly infested since the outbreak of 1953 and preventing reinfestation through aggressive control and regulatory action. Long-range plans call for progressive reduction of the infested area by means of expanded spray programs aimed at eventual eradication of the pest.

Favorable Congressional action on requests for funds in the spring of 1956 permitted an aggressive beginning to an accelerated program of gypsy moth elimination. The funds thus provided, in combination with appropriations by cooperating States, resulted in the spraying of more than 941,000 acres of infested territory during May and June. The principal area of activity by Federally contracted aircraft was in a rectangular shaped area containing 587,500 acres in contiguous sections of New York, New Jersey and Pennsylvania. More than 89,000 acres were sprayed cooperatively in sections of eastern New York where outbreak conditions were prevalent and a high hazard of larval spread by wind currents existed. In Michigan 98, 860 acres were aerially sprayed under State contract with Federal assistance. In all cooperative Federal-State operations DDT was applied at the rate of 1 pound in 1 gallon of spray per acre. Of the 155,000 acres sprayed by State agencies in New England to control the insect, the largest operation was in Massachusetts where approximately 120,000 acres were aerially treated.

Immediately following completion of the spray program, the most extensive gypsy moth trapping survey conducted to date was inaugurated. Nearly 25,000 traps were used in surveying an over-all area of about 10 million acres. Aside from the 1½ million acres trapped in Michigan, a major portion of the survey, approximating 7½ million acres, was conducted in a continuous belt of territory extending from Lake Ontario through central New York, northeastern Pennsylvania and northern New Jersey. A limited amount of checking with traps was carried on by cooperators in Ohio, Indiana, Virginia and West Virginia; all with negative findings. Traps were furnished the Canadian Department of Agriculture for checking in an area approximately 30 miles wide by 6 miles deep along the New York and Vermont State borders. Of 300 traps used in the Canadian survey, 51 captured 64 specimens identified as male gypsy moths. Traps were also distributed in northern New England to check on conditions outside the regulated area. In this section some extension of infestation was detected, especially in northwestern Vermont.

In the extensive belt of territory trapped in central New York, moths were captured at scattered locations outside the quarantined area. In no instance, however, was recovery made in a township at the western edge of the zone under survey. A concentration of catches occurred in a section of eastern Chenango County in territory adjoining a similar concentration in the quarantined county of Otsego. Other captures outside the regulated area were so widely separated as to encourage a belief that the objective of finding the outer limits of infestation has been virtually accomplished by this survey.

In Pennsylvania, traps that captured moths were located principally in towns adjacent to the Delaware River which forms the State boundary with New York and New Jersey. Here, also, no moths were captured in the western part of the trapped area. Moth recoveries in Pennsylvania show a southward extension of infestation that is associated, apparently, with the recent spread to this general area. This southward extension of infestation coincides with similar findings across the Delaware River in New Jersey. In neither State, however, were moths recovered to the southern extremity of the trapped area.

In New Jersey moths were captured in 27 of the nearly 2,000 traps distributed in the northern part of the State. In each of 25 attracting traps only a single moth was captured.

CURRENT STATUS OF INFESTATION.—Although a scouting survey to delimit outlying infestations is barely under way, a sufficient number of observations have been made by State and Federal field workers to obtain a general picture of infestation conditions. Within the regulated area, epidemic conditions are prevalent at scattered locations along the New York-New England border extending northward from territory directly east of Poughkeepsie, New York to sections in the Champlain Valley south of Burlington, Vermont. A relatively narrow strip of territory is affected by such conditions in each of the States involved.

In the older infested area of eastern New England there appears to be no extensive acreage in which outbreaks are imminent. In this area a high degree of larval mortality occurred in the summer of 1955 as the result of disease outbreaks caused by a polyhedral virus. The moth is not sufficiently recovered from this set-back to permit development of high populations in much of eastern New England. Large-scale control operations conducted each year since the 1953 outbreak also have reduced greatly the incidence of epidemic conditions in this area.

A high proportion of territory recently brought under quarantine regulations is lightly infested. Only in the eastern or Hudson Valley portion of this area is there evidence of general, continuous infestation and the rapid development of high populations.

Beyond the limits of the quarantined area, observations show that where infestation can be found it is of light intensity and generally confined to scattered, individual spots. Conditions in this respect are similar in New York, New Jersey and Pennsylvania.

PLANS FOR 1957.—A greatly expanded spray program is planned for the spring of 1957. Priority will be given to needed operations in partially infested States located beyond the limits of the quarantined area. This will include cooperative treatments in Michigan, New Jersey and Pennsylvania, aggregating possibly 500,000 acres. Additional spraying, estimated at more than two million

acres will be concentrated along the periphery of the regulated zone, mostly in New York, for the purpose of reducing the over-all area in which general infestation is prevalent. Also, extensive control work will be conducted in sections of the quarantined area where epidemic conditions occur.

Control operations in 1957 will be followed immediately by a large-scale trapping program. Traps will be distributed beyond the limits of past surveys as well as in recently sprayed territory to check on effectiveness of treatments applied. Such trapping will be necessary on a continuing schedule as a means of determining where additional treatments may be required in the mop-up phase of the eradication effort.

The Western Bean Cutworm as a Pest of Corn in Idaho[1]

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The western bean cutworm (*Loxagrotis albicosta* (Sm.)) is probably a native of North America and was described in 1887 from moths collected in Arizona. This insect is well distributed in the bean-growing areas of Colorado. According to Crumb (1956), it has been recorded from Arizona, Colorado, Idaho, Iowa, Kansas, Nebraska, New Mexico, Texas, and Utah, as well as from Mexico and Alberta, Canada. McCampbell (1941) and Hoerner (1948) reported on this cutworm as a pest of beans in Colorado, and Douglass *et al.* (1955) discussed its life history, habits, and control on beans in Idaho. Its feeding habits differ from those of the common garden cutworms that cut off the plants near the soil surface in the spring of the year. It belongs to the group of climbing cutworms that feed upon the foliage, stems, buds, seeds, or fruit. It also is known to feed upon the fruits of ground cherry and nightshade.

On August 24, 1954, while working in Idaho, R. E. Miller, an entomologist of the Green Giant Company of Dayton, Washington, brought to the Twin Falls, Idaho, field station some worms he found in corn grown by Leslie Goble near Tuttle, Idaho, for canning. These worms were identified as the western bean cutworm. The ears of corn in this field had been picked for processing before additional specimens could be found. Some of the ears and all of the nubbins that had been left by the picker were injured, and as many as seven worms were found in one ear. These worms are not cannibalistic like the corn earworm (*Heliothis zea* (Boddie)).

As cool weather approaches, the mature worms enter the soil to a depth of 3 to 11 inches, an average of 8½ inches, and construct earthen cells in which to pass the winter. When the soil warms up late in the spring or early in the summer, they change to pupae. Evidently moist soil, resulting from heavy rains or irrigation, is necessary for the moths to emerge from the pupal cells deep in the ground. The earliest emergence in hibernation cages at Twin Falls during the 1951–56 period occurred on July 3, 1956, and the latest on August 12, 1955. The average emergence dates for this period were July 12 for the first, July 22 for the peak, and August 1 for the last. This shows that the average emergence period is only 20 days. The peak of moth capture in light traps coincides with the peak of emergence in hibernation cages.

LIFE HISTORY.—Life-history studies were made in an insectary at Twin Falls during the 1956 season. A total of 86 female moths had preoviposition periods ranging from 1 to 5 and averaging 3 days. The oviposition period ranged from 2 to 13 and averaged 8 days. The number of eggs laid per female varied from 84 to 627 and averaged 321. The first moths emerged from hibernation on July 3, and by August 1 emergence and oviposition had been completed and practically all the moths had died. There was considerable overlapping of instars of larvae from the same egg mass, but the average time from hatching to the first molt was 5 days, with 4 days for the second, third, and fourth, and 5 days for the fifth. Larvae have been observed injuring corn from July 21 to October 3.

The eggs are laid on all exposed parts of the corn plant and hatch in 5 to 7 days. The young eat the eggshells before scattering over the plant. They move readily and, during the day, hide between the leaf sheath and stem, or stalk, of corn plants. The larvae from one egg mass create an infestation center, which gradually spreads out in a circle that may be 10 or more feet in diameter. The larvae feed on all parts of the plant, including the leaves, stems, tassels, shanks, husks (fig. 1), kernels (fig. 2), and cobs. They are very small when hatched, dark in appearance, and consume very little food during the first few days. After the larvae are a little larger, they feed through the leaves, and the injury resembles shot holes. These holes may be readily observed, especially in the outer husk, or "flags," of sweet corn. When the larvae have grown to about ½ inch in length, they start boring into the stem, shank, and ears. Their holes through the husk resemble the exit holes of the corn earworm from ears of corn. They generally enter the ear through the husk anywhere from the butt to the tip. As the kernels mature in the early ears, the larvae move down into nubbins and continue to feed until mature or until the kernels harden.

DISTRIBUTION AND DAMAGE IN IDAHO.—As a result of the finding of the western bean cutworm on corn in 1954, a survey of the distribution and damage by this insect in the Snake River and Boise Valleys of Idaho was made in 1955 by J. W. Ingram with the assistance and advice of the senior author. From August 3 to 17, 37 fields of dent corn and 18 of sweet corn between Rupert and Glenns Ferry, Idaho, were examined. Only six fields of the dent corn and one of the sweet corn were infested. One of the infested fields was in the whorl, two were in the tassel, and four in the silk stage of growth. One egg mass and one

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[2] W. N. Oliver and W. A. McIntyre assisted in some of these investigations

FIG. 1.—Corn showing damage to leaves, stalks and ears caused by feeding of western bean cutworm.

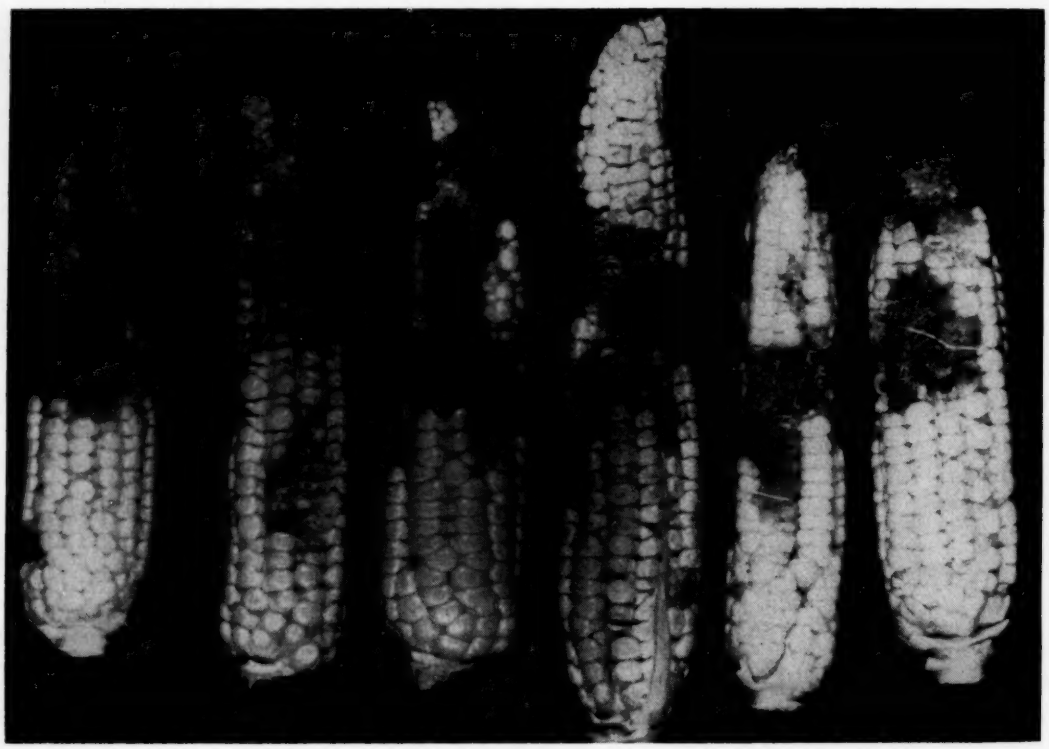

FIG. 2.—Corn ears showing severe damage caused by the western bean cutworm.

moth were found in the whorls. About 30 minutes were spent examining each field. In the most heavily infested field five infested plants were found. This study was made too early in the season to get an accurate estimate of the situation, as the peak of adult emergence did not occur until July 28 and larvae probably would not have been large enough to be readily observed until August 15 or later. The infestation is more readily detected later in the season after the worms are large.

A similar study was made in the same general area from August 2 to September 26, 1956, in which 49 fields of sweet corn and 37 of dent corn were examined. The western bean cutworm was found in 30 fields of sweet corn and 23 fields of dent corn. During the first half of August, the infestation was confined largely to early fields on sandy loam soil, but during the latter part of September the heaviest populations were found in late corn. At that time typical bean cutworm injury was noted in nine fields, but no worms were found. Apparently they had reached full growth and had entered the soil for the winter. On August 2 large worms were found in sweet corn in the Rupert area, and on August 11 in a field of sweet corn in Mellon Valley northeast of Buhl, Idaho, 12.5% of the ears had side-entry holes. The distribution in Idaho in 1956 extended from east of Rupert to west of Glenns Ferry, a distance of more than 100 airline miles.

In the Magic Valley of southcentral Idaho, dent corn is grown commercially for ensilage and sweet corn for canning under contract and for seed. Most of the sweet corn grown for canning is cut for ensilage after the ears have been picked. The average length of the growing season at Twin Falls is 132 days, but killing frost has occurred as late as July 14 and in the fall as early as August 31. Therefore, it is very unlikely that planting dates can be so timed that the crop will escape injury. Planting of corn for both canning and seed is spread over as long a period as the season will permit in order to extend the harvest to facilitate orderly handling of the crops. Some of the corn is cut for ensilage before the worms enter the soil for overwintering, and this practice helps reduce the populations.

The western bean cutworm has not yet been found in the Boise Valley of western Idaho, which produces approximately 85% of the hybrid sweet corn seed used in the United States.

So far this cutworm has been a more serious pest of beans and corn on sandy loam than on heavier soils.

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DDT Accumulation in Mid-Western Orchard and Crop Soils Treated Since 1945[1,2]

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Since the appearance of synthetic organic insecticides and their widespread use in agriculture, residue problems have increased more and more in importance. DDT has now been used over 10 years on various agricultural crops. In many cases this insecticide has been applied in orchards or on annual crops at such rates that disappearance of the residue from the soil was unable to keep up with application and a gradual accumulation has occurred (Chisholm *et al.* 1951, Fleming *et al.* 1951, Ginsburg & Reed 1954, Ginsburg 1955, Koblitzky & Chisholm 1949). Moreover, the possible influence of breakdown products of the chemical on crops presents an increasingly serious problem. It is, therefore, of importance to know how much residue is present in a certain soil type in order to guide the farmer when replanting these soils with various crops.

PROCEDURE.—In the fall of 1955, soil samples were collected from 14 orchard soils in Missouri, Indiana, Ohio and Michigan, from 24 crop soils in Wisconsin, Iowa, Illinois, North Dakota, and Missouri, and from three turf plots in Ohio. DDT had been applied to the trees and crops according to the usual spray applications between the years of 1945 and 1955, sometimes resulting in yearly applications over a period of 11 years.

Sampling. In orchard soils sampling was confined to the soil under the trees after the grass and litter had been pushed aside in order to obtain soil only. Five random cores ($\frac{3}{4}'' \times 6''$) of soil were collected under each of 10 trees scattered throughout the entire orchard or uniformly treated block resulting in a total of 50 cores. Untreated samples were collected outside the orchard but from comparable soil types.

In crop and turf soils 50 cores were collected from all parts of the field completely at random. The 50 "control" cores were taken from adjoining areas, although in some cases it was extremely difficult to secure uncontaminated soils. The collected soil samples were then placed in plastic bags, closed and shipped in ice cream cartons to the University of Wisconsin for screening, mixing, extraction, and analysis.

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Table 1.—DDT recovered in 1955 from upper 6″ of silty clay loam turf plots in Cleveland, Ohio, treated in 1945 only.

DDT Applied	DDT Recovered		
Lbs./Acre	P.P.M.	Lbs./Acre	Per Cent of Amount Applied
12.5	0.77	1.36	10.9
25.0	2.04	3.52	14.1
37.5	3.89	6.72	17.9

Methods.—After screening and mixing, soil samples were extracted with a mixture of benzene-isopropanol (2:1), cleaned up, by shaking with a mixture of filter cel and decolorizing carbon (4:2) and subsequently analyzed according to the specific analytical method as proposed by Schechter & Haller (1945), and abridged by Pontoriero & Ginsburg (1953). The results obtained are expressed as technical DDT in the tabulated results given below. No correction was made for overage in insecticide formulations. Finally, results were expressed in parts per million based on the dry weight for each soil type, and in lbs./6″-acre after the dry weight of each 6″-acre had been calculated on the basis of the dry weight of fifty 6″ core samples.

RESULTS.—*DDT in Ohio-turf plots.*—One of the most interesting findings concerning the long persistence of DDT in soil was revealed by the investigation of soil samples from Cleveland, Ohio (table 1). Those turf plots, a silty clay loam, were treated in 1945 with dosages of 12.5 lbs./acre, 25 lbs./acre and 37.5 lbs./acre of DDT for Japanese beetle control. During the next 10 years no additional DDT was applied, yet 10.9%, 14.1% and 17.9% of the original application of DDT were found to be present in the upper 6″ layer. As we have found in other investigations, the percentage loss of the insecticide tended to be inversely proportional to the original dosage.

DDT recovered from apple and peach orchards.—The largest amounts of DDT were recovered from orchard soils (table 2) where high volume spraying for the control of orchard insects resulted in an appreciable accumulation of residual DDT in the soil. Altogether, soils from 9 apple orchards and 5 peach orchards were investigated; one apple orchard received DDT for 11 years, two others were sprayed with DDT for 9 years, while the remaining six had a 10-year accumulation of the insecticide. Two peach orchards received DDT for 10 years while the remaining three had 3- to 6-year deposits of this particular insecticide.

The DDT recovered from the soil amounted to 93.5–106 p.p.m. in Indiana, 38.6 p.p.m. in Ohio, 36.6 p.p.m. in Missouri and 1.5–38.3 p.p.m. in Michigan. The average recovery for all 14 orchards investigated was 26.6% of the total amounts applied and 237.0% of the average yearly application.

DDT recovered from crop soils.—The amount of DDT found in the upper 6″ layer of crop soil was considerably less than in orchard soils (table 3). Altogether 24 soils were analyzed, 22 of which contained DDT ranging from 0.38 to 4.6 p.p.m. The amounts recovered were 0.53–0.90 p.p.m. in Wisconsin (corn), 0.56–1.0 p.p.m. in Iowa (corn, oats and alfalfa), 0.76–2.22 p.p.m. in the East St. Louis area of Illinois (corn, horseradish, potatoes), 0.6–3.25 p.p.m. in the Rochelle area of Illinois (corn, lima beans, peas, barley and pumpkin) 1.46–4.6 p.p.m. in the DeKalb area of Illinois (corn, peas, barley, lima beans, oats), 0.38–0.55 p.p.m. in North Dakota (potatoes, wheat, flax, barley) and 0.49 p.p.m. in Missouri (potatoes).

The average recovery from all 24 crop soils investigated resulted in 15.5% of the total amount applied and 61.2% of the average yearly application.

SUMMARY.—A survey was made on the accumulation of DDT in soils of midwestern orchards and crop soils, treated with DDT for insect control from 1945 to 1955.

Table 2.—DDT recovered from upper 6 inches of soil from orchards treated between 1945 and 1955.

Location	Soil Type	DDT Applied		DDT Recovered		
		Years	Total Lbs./Acre	P.P.M.	Lbs./Acre	Per Cent of Total Applied
		Apple Orchards				
Missouri						
Independence	Marshall silt loam	1945–55	562	36.6	61	10.8
Indiana						
Vincennes	Princeton fine sandy loam	1946–55	240	106	197	82.0
Vincennes	Alfred & Princeton silt loam	1946–55	245	93.5	173	70.5
Ohio						
Lorain	Sandy loam	1946–55	225	38.6	62.2	27.7
Michigan						
Lansing	Hillsdale clay loam	1946–55	99	5.7	10.5	10.6
Ludington	Sandy loam	1946–55	175	24.8	51.8	29.6
Lansing	Hillside clay loam	1946–55	100	6.2	12.3	12.3
Grand Rapids	Miami silt loam	1947–55	133.4	1.5	3.0	2.2
Benton Harbor	Coloma sand	1947–55	360	38.3	70.0	19.5
		Peach Orchards				
Ludington	Sandy loam	1946–55	197	17.3	35.8	18.2
Benton Harbor	Coloma sand	1946–55	346	12.5	26.2	7.6
Grand Rapids	Emmet clay loam	1950–55	98	9.4	17.1	17.5
Traverse City	Emmet sandy loam	1950–55	72	11.3	24.4	34.1
Niles	Miami silt loam	1953–55	60.2	10.3	18.2	30.3

Table 3.—DDT recovered from upper 6 inches of crop soils treated between 1946 and 1955.

Location	Soil Type	Crops Grown	DDT Applied		DDT Recovered		
			Years	Total Lbs./ Acre	P.P.M.	Lbs./ Acre	Per Cent of Total Applied
Wisconsin							
Oakfield	Miami silt loam	Corn	1949–50, 1952–55	16.5	0.53	0.73	4.5
Rosendale	Carrington loam	Corn	1950–52, 1954–55	16.0	0.90	1.21	7.6
Iowa							
Ankeny	Clarion loam	Corn, oats, alfalfa	1952	3.0	0.56	0.96	32.0
Ankeny	Clarion loam	Corn, oats, alfalfa	1953	3.0	0.77	1.28	42.6
Ankeny	Clarion loam	Corn	1955	3.0	0.80	1.37	45.6
Ankeny	Webster loam	Corn	1954–55	6.0	1.0	1.73	28.7
Illinois							
E. St. Louis	Sandy loam	Corn, horseradish	1950–55	36.0	2.22	4.12	11.5
E. St. Louis	Sandy loam	Corn, horseradish	1950–55	34.5	1.14	2.12	6.1
Collinsville	Sandy loam	Corn, horseradish, potatoes	1945–55	32.0	1.03	1.93	6.1
Collinsville	Sandy loam	Corn, horseradish, potatoes	1945, 1947–55	42.6	0.76	1.44	3.4
Rochelle	Brown silt loam	Sw. corn, lima beans, peas	1946–49, 1952, 1955	21.0	2.48	4.51	20.5
Rochelle	Black silt loam	Sw. corn, lima beans, grain, peas	1946–48, 1951–54	24.0	3.25	5.9	24.6
Rochelle	Black silt loam	Corn, barley, peas, pumpkin, lima beans	1946, 1948, 1949, 1953, 1955	12.0	1.36	2.48	20.7
Rochelle	Black silt loam	Corn, barley, peas, pumpkin, lima beans	1946, 1948, 1949, 1952, 1953, 1955	14.25	0.6	1.08	7.6
Rochelle	Black silt loam	Corn, peas, lima beans	1947–50, 1955	12.0	0.73	1.32	11.0
Rochelle	Brown silt loam	Corn, lima beans, peas	1946–49, 1951–55	24.0	2.62	4.8	20.0
De Kalb	Brown silt loam	Corn, peas, barley, lima beans	1946–48, 1953, 1955	16.5	0.0	0.0	0.0
De Kalb	Brown silt loam	Corn, peas, oats	1946–49, 1954, 1955	28.5	4.6	7.75	27.2
De Kalb	Brown silt loam	Corn, lima beans, peas	1946, 1948–50, 1952, 1953	15.0	1.46	2.46	16.4
N. Dakota							
Grand Forks	Silty clay loam	Potatoes, wheat, barley	1949, 1952, 1955	7.25	0.55	0.79	10.9
Grand Forks	Silty clay loam	Potatoes, wheat	1950, 1953, 1955	7.25	0.0	0.0	0.0
Grand Forks	Glyndon loam	Potatoes, wheat, flax, barley	1948, 1950, 1954	6.0	0.47	0.73	12.2
Grand Forks	Glyndon loam	Potatoes, wheat, barley, flax	1947, 1951	6.75	0.38	0.6	8.9
Missouri							
Courtney	Sarpy fine sandy loam	Potatoes	1947–55	22.5	0.49	0.9	4.0

Soil samples were collected in the fall of 1955 and analyzed for DDT by the Schechter-Haller method. In orchard soils, 93.5–106 p.p.m. were recovered in Indiana, 38.6 p.p.m. in Ohio, 36.6 p.p.m. in Missouri and 1.5–38.3 p.p.m. in Michigan.

In crop soils, 0.53–0.90 p.p.m. were recovered in Wisconsin, 0.56–1.0 p.p.m. in Iowa, 0.6–4.6 p.p.m. in Illinois, 0.38–0.55 p.p.m. in North Dakota and 0.49 p.p.m. in Missouri. No relationship was established between the amount of DDT found in the soil and the various crops grown in these soils. Turf plots in Ohio, treated with one application of DDT in 1945 only, contained 10.9%–17.9% of the original application, when analyzed in 1955.

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The Relative Toxicity of DDT, Toxaphene, Lindane and Isodrin to *Dendroctonus brevicomis* Lec. and *Ips confusus* (Lec.)[1]

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Bark beetles are by far the most serious insect pests of western forests. About 2.8 billion board-feet of western timber is killed by these insects every year (Keen 1952), and in some years epidemics of one or more species have increased this loss several fold. At present, it is necessary to resort to one of two general methods of direct control to suppress outbreaks of these insects: burning the infested host or treating infested trees with a penetrating oil spray. Both of these measures are expensive and limited in applicability. Fire, of course, is a very hazardous tool, and its use is limited to areas or seasons of the year in which it can be employed safely. Use of penetrating oil sprays is limited by the size and accessibility of the infested trees. Relatively large quantities of spray are needed. If the trees are large and not easily accessible, the cost of transporting the spray alone is almost prohibitive.

Because of these limitations, investigations were started to determine the potentialities of residual-type insecticides for both direct and preventive control of bark beetles. It was felt that it might be possible to coat the outer bark surface of the trees with an insecticide deposit that would: (1) kill the adult beetles as they emerge from infested trees or (2) prevent attacks on valuable uninfested trees.

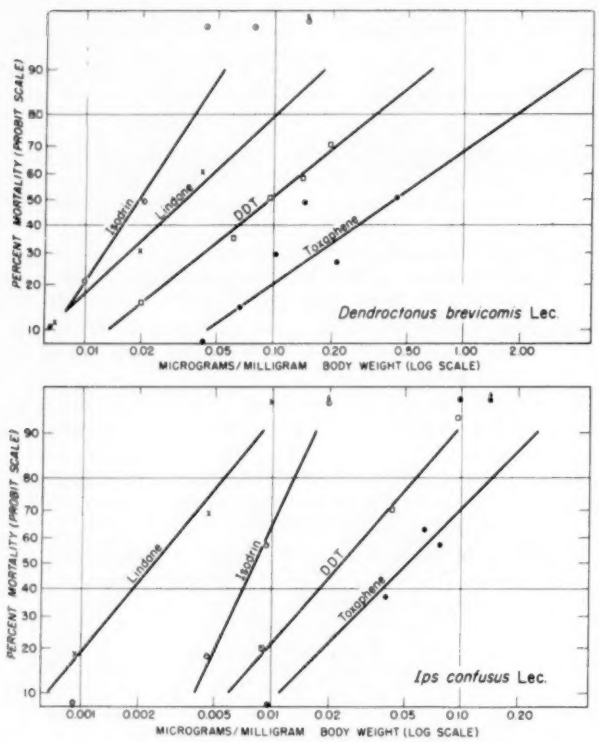

FIG. 1.—Dosage-mortality relationship obtained by topical application of DDT, toxaphene, lindane, and isodrin (in acetone) to the thoracic sterna of adults of *Dendroctonus brevicomis* Lec. and *Ips confusus* (Lec.)

Because the beetles would contact the insecticide only briefly, effective treatment would have the following requirements: the insecticide must be very toxic to the beetle species in question; it must be evenly distributed over the bark so that the insect will come in contact with it; and the deposit must be in such a physical state that a lethal dose can be readily picked up.

Much of the information needed to obtain even insecticide deposits is already available, but there is almost no information on the relative toxicity of the insecticides that might be considered for this purpose, nor on the factors influencing the physical state and availability of deposits on bark. Therefore, appraisal of the potential toxicity of a number of candidate insecticides was chosen for study first. The studies reported here were carried out during the summer of 1953 at a U. S. Forest Service field laboratory on the Sierra National Forest in California. The insecticides compared were DDT, toxaphene, lindane, and isodrin. The test beetles were the western pine beetle (*Dendroctonus brevicomis* Lec.), and the California five-spined engraver beetle (*Ips confusus* (Lec.)). The studies will be continued with other insecticides and later supplemented by studies on the physical state, availability and residual life of insecticide deposits on bark.

MATERIALS AND METHODS.—Toxicity was determined by observing the mortality of newly emerged adults treated with microquantities of varying concentrations of the test insecticides. Dosage mortality relationships and relative potencies were determined by the probit method (Cochran 1938, Finney 1952).

The test beetles were obtained by caging infested log samples in a compartmented outdoor insectary and collecting the adults as they emerged. Both *D. brevicomis* and *I. confusus* were reared from ponderosa pine obtained from natural infestations within a 30-mile radius of the field laboratory.

All insecticide solutions were prepared with acetone. During preliminary investigations to determine the approximate concentration of insecticides that would have to be used to work within the 0 to 100 percent mortality range, every other check sample was treated with acetone to determine any deleterious effect this solvent might have. There was no significant difference in mortality between those test groups treated with acetone and those left untreated.

[1] The studies reported are part of a research program being conducted by the California Forest and Range Experiment Station, U. S. Forest Service, and the Department of Entomology and Parasitology, University of California, on the control of bark beetles with residual-type insecticides. Accepted for publication March 12, 1957.

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Table 1.—Mortality of adults of *Dendroctonus brevicomis* and *Ips confusus* at varying doses of DDT, toxaphene, lindane, and isodrin, 72 hours after treatment.

CONCENTRATION (MG./100 ML.)	NUMBER OF TEST SUBJECTS	NUMBER DEAD AFTER 72 HRS.	PER CENT MORTALITY[a]	MORTALITY CORRECTED FOR CHECK
	Dendroctonus brevicomis Lec.			
	Untreated Checks			
0.0	219	2	1	—
	DDT			
10.0	80	14	17	16
30.0	40	14	35	34
50.0	120	63	52	52
70.0	40	23	57	57
100.0	80	56	70	70
	Toxaphene			
20.0	80	7	9	8
30.0	20	3	15	14
50.0	20	6	30	29
70.0	20	10	50	49
100.0	80	22	28	27
200.0	80	40	50	49
	Lindane			
2.0	80	6	7	6
10.0	100	32	32	31
20.0	80	47	59	59
	Isodrin			
5.0	97	22	23	21
10.0	100	49	49	48
20.0	20	19	95	95
40.0	20	19	95	95
80.0	20	20	100	100
	Ips confusus (Lec.)			
	Untreated Checks			
0.0	200	9	5	—
	DDT			
5.0	100	24	24	20
25.0	100	73	73	72
50.0	100	92	92	92
	Toxaphene			
4.0	120	15	12	7
20.0	100	40	40	37
30.0	20	13	65	63
40.0	120	67	56	54
50.0	20	19	95	95
70.0	20	20	100	100
	Lindane			
0.5	100	22	22	18
2.5	100	70	70	68
5.0	100	94	94	94
	Isodrin			
0.5	100	13	13	8
2.5	100	22	22	18
5.0	120	68	57	55
10.0	20	20	100	100

[a] To nearest whole number.

The purity of the products used to prepare the test solutions was as follows:

DDT[4] Pure p-p′ isomer (twice recrystallized from tech. grade), 2,2-bis (p-chlorophenyl)-1,1,1-trichloroethane

Toxaphene[5] 100% tech. grade, chlorinated camphene of undetermined molecular structure.

Lindane[4] 99% gamma isomer, 1,2,3,4,5,6-hexachlorocyclohexane.

Isodrin[6] 91%, 1,2,3,4,10,10-hexachloro-1,4,4a,8,8a-hexahydro-1,4,5,8-endo-endo-dimethanonaphthalene

The test solutions were held in dropping bottles with frosted glass stoppers, and fresh solutions were prepared every 2 to 4 days. This 2- to 4-day use-period apparently was not critical because no appreciable evaporation could be detected in a marked series of check bottles until after 2 weeks.

The test solutions were applied to the beetles with a 0.25-ml. tuberculin syringe manipulated by means of a micrometer. The spindle stop and $\frac{1}{2}$ inch of the micrometer frame were removed and a clamp mounted at this point to receive the syringe. The clamp was mounted so that the plunger of the syringe fell in direct line with the micrometer spindle and could be actuated by manipulating the spindle. The syringe used was fitted with a 26-gauge hypodermic needle bent to a 90° angle and blunted for topical application.

Table 2.—Toxicity of DDT, toxaphene, lindane and isodrin to *Dendroctonus brevicomis* and *Ips confusus*.

INSECTICIDE	*D. brevicomis*	*I. confusus*
	LD_{50} (μg./mg. body wt.)	
DDT	0.095±0.010[a]	0.024±0.002
Toxaphene	0.428±0.095	0.049±0.008
Lindane	0.034±0.005	0.002±0.000 (2)
Isodrin	0.019±0.003	0.008±0.000 (5)
	LD_{90} (μg./mg. body wt.)	
DDT	0.682±0.215	0.093±0.013
Toxaphene	4.303±2.575	0.255±0.104
Lindane	0.182±0.068	0.009±0.001
Isodrin	0.054±0.020	0.017±0.002

[a] Standard Error.

All live beetles were removed from the rearing cage at periodic intervals. A record was kept of the time of collection, the time lapse between collections, and the time lapse between collection and treatment. From this information, the possible range in time between emergence and treatment was computed for each test batch. Test beetles were held in petri dishes 0 to 4 hours before treatment. Twenty adults were removed from each test batch to serve as checks. All beetles were handled with a dry or slightly moist camel's-hair brush to avoid traumatic injuries.

Each test beetle was placed on its back on the stage of a dissecting scope. The micrometer spindle was turned one full unit (50 divisions) and the drop produced removed with cotton. This precluded the danger of applying a more concentrated solution than desired, which would result from evaporation at the needle tip. A second droplet, of the desired volume, was produced at the needle tip and brought in contact with the thoracic sternal region of the beetle. The volume applied was directly proportional

[4] Contributed by Stauffer Chemical Company, Los Angeles, California.
[5] Contributed by Hercules Powder Company, Wilmington, Delaware.
[6] Contributed by Julius Hyman & Company, Denver, Colorado.

Table 3.—Relative potency of DDT, toxaphene, lindane and isodrin to *Dendroctonus brevicomis* and *Ips confusus*.

Insecticide	*D. brevicomis*		*I. confusus*	
	Relative Potency	Fiducial Limits (95% Probability)	Relative Potency	Fiducial Limits (95% Probability)
Toxaphene	0.24 ±0.05[a]	0.14–0.34	0.46±0.06	0.34– 0.58
DDT	1.00 (standard)		1.00 (standard)	
Lindane	2.67 ±0.47	1.74–3.59	9.83±1.35	7.18–12.48
Isodrin				
at LD_{50}	4.99 ±1.01	3.01–6.97	2.84±0.35	2.15– 3.53
at LD_{90}	12.59(±6.21)	—	5.35±1.04	3.31– 7.39

[a] Standard Error.

to the average weight of the test subjects of each species.

Usually three concentrations of an insecticide were tested at one time. With the exception of DDT, the insecticides included in each test series were varied so that not all concentrations or replications of any one toxicant would be run at the same time. DDT was included in all test series.

Each treated and check beetle was placed in a gelatin capsule and removed to a basement room where the temperature remained 16° to 21° C. and the relative humidity between 70 and 80%. Mortality was observed 72 hours after treatment. Motionless insects were removed from the capsules and probed with a needle. All individuals that did not respond to this stimulus were considered dead.

Results.—In the first tests, the beetles were removed from the rearing cages twice daily, and the possible range in time between emergence from the host and treatment was 0 to 24 hours. To reduce this time lapse, beetles were collected more frequently in subsequent studies, and those that emerged between the last collection of 1 day and the first collection of the next day were excluded from the tests. The results were then separated into two classes: (1) tests in which the beetles were treated within 12 hours after emergence, and (2) tests in which treatment was as much as 24 hours after emergence. The average natural mortality 72 hours after treatment for these two groups was as follows:

Hours between emergence and treatment	*Per cent mortality*	
	Dendroctonus	*Ips*
0 to 12	1	5
0 to 24	16	15

To avoid an unduly large variance, only those tests in which all beetles were treated within 12 hours after emergence were included in the final analysis of the data. The number of beetles included in each treatment and the resultant mortality are given in table 1.

All insecticides were more toxic to *I. confusus* than to *D. brevicomis* (table 2).

The order of toxicity of the test toxicants was (fig. 1):

D. brevicomis—isodrin>lindane>DDT>toxaphene
I confusus—lindane>isodrin>DDT>toxaphene

The relative potencies of the test toxicants in relation to DDT (selected as a standard) are given in table 3. There was no significant difference in parallelism between the regression lines for DDT, toxaphene, and lindane, but a very significant difference between the slope of the regression lines for these toxicants and that for isodrin. Therefore, it was necessary to determine the relative potency of isodrin at definite dosage levels. Both the LD_{50} and LD_{90} levels were used for this purpose, but the standard error of the LD_{90} estimate for *D. brevicomis* was so high that this value contributed little to the evaluation.

Summary.—The toxicity studies reported here are part of a general research program on the control of bark beetles with residual-type insecticides. The objective is to develop a method of control that (1) will prove cheaper and more satisfactory than burning or use of penetrating oil sprays and (2) will provide a means of protecting individual trees of high value from beetle attack. As a first step, toxicity indices were determined for some promising residual-type insecticides. A satisfactory screening procedure was developed, and tests were conducted on the relative toxicity of DDT, toxaphene, lindane, and isodrin to *Dendroctonus brevicomis* Lec. and *Ips confusus* (Lec.). The more important results obtained were as follows:

The order of toxicity of the test toxicants was:

D. brevicomis—isodrin>lindane>DDT>toxaphene
I. confusus—lindane>isodrin>DDT>toxaphene

All of the test insecticides were more toxic to *I. confusus* than to *D. brevicomis*.

For both species, a parallel regression relationship was obtained between DDT, toxaphene and lindane, while a significantly steeper slope was obtained for isodrin.

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Use of a Polyhedrosis Virus to Control the Cabbage Looper on Lettuce in California[1,2]

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The cabbage looper, *Trichoplusia ni* (Hbn.), which is a major pest of crucifers and many other plants throughout the United States, is an important pest of lettuce in the desert valleys of the Southwest, where the young plants of the fall crop often are subjected to severe attack and the maturing heads frequently are seriously infested. McKinney (1944) reports the presence of unidentified diseases in loopers on lettuce in Arizona, with little evidence of microbial control of the pest except during periods of excessive rainfall.

The looper also occurs on cotton during the summer months in the desert areas, where occasional outbreaks often are followed by widespread epizootics of a polyhedrosis virus disease. Since there are no records of epizootics caused by naturally occurring virus in looper populations on lettuce, the studies reported herein were initiated to determine the potential value of the polyhedrosis virus which attacks the looper so effectively on cotton as a biological agent to control the looper on lettuce.

The occurrence of a virulent disease of the cabbage looper in the United States has been known for many years. A review of the early reports of this disease is given by Semel (1956). The polyhedrosis virus was reported first as such by Chapman & Glaser (1915). Steinhaus (1949) mentions that the disease has also been observed in Russia. The virus is known to occur in many parts of the United States (Williams 1923, Walker & Anderson 1936, Hayslip *et al.* 1953, Semel 1956). In the Southwest (Arizona and California) epizootics have been observed frequently in looper populations on various cruciferous crops as well as on cotton.

Virus-infected cabbage loopers display marked symptoms. On the third to fifth day after infection the larvae begin to feed less and become less active. The integument becomes more pale and, in the larger larvae, rather blotchy. The blotchy appearance is caused by the opaque-white fat bodies showing through the transparent integument. Many of the older larvae, which often survive for several days, also develop irregular dark spots on the integument.

Shortly before death, the larvae cease to move and the integument becomes very shiny. The epidermis and body contents of many of the small larvae often turn bright yellow. Some of the larvae die when in a normal, upright position, although the characteristic sac hanging by the prolegs occurs commonly. When pricked, these upright bodies collapse into a fluid mass. Shortly after death, the intact body and enclosed fluids begin to darken, becoming first gray, then brown, and finally black. With evaporation of the fluids, the remains dry to a black scale.

A number of infected mature larvae were dissected and the various tissues were examined for the presence of polyhedra. Only the adipose tissues were found to harbor polyhedral bodies. The fat cells were greatly hypertrophied and were packed with masses of polyhedra. Because of this, the fat tissues were quite opaque and appeared to be white in color when dissected from the body cavity of the larva.

Production of virus material for laboratory and field studies was first accomplished by rearing naturally infected mature loopers collected from a cotton field near Blythe, California. At the time of collection, some signs of larval death caused by virus were noted in the field, and almost all the larvae died of the infection within a few days after being brought into the laboratory. Additional material was obtained by collecting the remains of larvae killed in virus-infectivity tests.

LABORATORY STUDIES.—A series of laboratory studies was undertaken to determine the effect of the virus against insectary-reared cabbage loopers. In preparation for conducting these tests, a stock of disease-free insects was started. To prevent the appearance of the virus in the insectary stock, all the looper eggs were placed in a bath of 10% formaldehyde for 1½ hours and then thoroughly washed in tap water for at least 10 minutes. The insects used to maintain the insectary population were individually reared in steam-sterilized half-pint cartons with petri dish tops. The insects used in the tests were transferred to similar sterilized units shortly after hatching and were reared in these units until the tests were terminated.

The *Trichoplusia* larvae were reared on a variety of plant material. When available, young cotton leaves were utilized. At other times, the loopers were fed cabbage, lettuce, or alfalfa.

A series of infectivity tests was conducted to determine the virulence of the Blythe-collected cabbage-looper virus for second- and fourth-instar larvae. Three concentrations of the virus (determined by haemacytometer counts of polyhedra) were used in the tests: 10 million, 1 million, and 100 thousand polyhedra per ml. In the first experiment against early-instar larvae, each virus concentration was tested against 25 larvae set up in groups of 5 larvae per sterile carton. When more insects became available, the experiment was repeated, with each concentration tested against 100 larvae set up in groups of 10 per sterile carton. In the test against older loopers, 50 larvae were set up in groups of 5 per carton. In each test a similar number of larvae were maintained as untreated controls. When each test was set up, the control larvae were fed uncontaminated plant material, while the test insects were fed plant material which had been dipped in one of the standardized suspensions of the virus. Each group was reared at room temperature (about 22 °C.). Results of counts made of living larvae on the third, fifth, seventh, and ninth days after the start of each test are presented in table 1.

Semel (1956) reports that the last larval stages were

[1] Contribution of the Laboratory of Insect Pathology, Department of Biological Control.

[2] Paper No. 948, University of California Citrus Experiment Station, Riverside, California. Accepted for publication March 12, 1957.

[3] Assistant Insect Pathologist, Department of Biological Control.

Table 1.—Mortality of early- and late-instar larvae of *Trichoplusia ni* (Hbn.), subjected to various concentrations of polyhedrosis virus.[a]

Virus Concentration (Polyhedral/Ml.)	Larval Instars at Start	No. of Larvae Tested	Per Cent Mortality[b] (Days After Treatment)			
			3	5	7	9
1×10^7	1st and 2nd	125	0	59	90	100
	4th and 5th	50	0	51	55	100
1×10^6	1st and 2nd	125	2	27	72	93
	4th and 5th	50	0	2	9	100
1×10^5	1st and 2nd	125	0	7	56	85
	4th and 5th	50	0	0	0	84

[a] Tests conducted at room temperatures averaging 22° C. (72° F.).
[b] Cumulative (corrected by Abbotts' formula).

more susceptible to the disease than the earlier larval stages, because only rarely were infected early-instar larvae observed in the field. Results of the present infectivity tests indicate, however, that under moderate temperatures small larvae are more easily killed by the virus than are large larvae, although mortality is equally high in both groups after about 9 days. Treatments with less concentrated virus suspensions extend the incubation period of the disease in larvae of both age groups. This is especially noticeable in the group of older larvae, where mortality was quite low through the seventh day.

Further tests were conducted to determine the effect of the virus on cabbage loopers reared at sustained low temperatures. For these tests, three standard refrigerators were regulated to temperatures of 15.5° C. (60° F.), 10° C. (50° F.), and 4.4° C. (40° F.). The relative humidity in each cabinet was adjusted to about 75% by the use of solutions saturated with rock salt.

First-instar larvae were set up in units of five per sterile carton. Twenty cartons were placed in each refrigerator, 10 to serve as controls and 10 to be utilized in the infectivity tests. The control larvae were fed uncontaminated alfalfa sprigs, while the test larvae were fed alfalfa sprigs which had been dipped in a suspension of the virus (concentration, 1×10^7 polyhedra per ml.) with a small amount of blood albumin spreader added.

Counts of living larvae in each test and control group soon revealed that small larvae of *Trichoplusia ni* are not able to survive sustained temperatures of 4.4° C. This portion of the experiment was quickly closed out, and counts were continued in the 10° C. and 15.5° C. groups until death of the last test larva. The results (table 2) give an indication of the importance of temperature in controlling the activity of the virus. Whereas 100% mortality was noted on the ninth day in infected test larvae reared at a temperature of about 22° C. (see table 1), the data presented in table 2 indicate that a temperature reduction of 6.5 degrees centigrade more than doubled the time required for 100% mortality. A further reduction of 5.5 degrees centigrade resulted in more than a five-fold increase in the time required for all the test larvae to die.

Table 2.—Effect of sustained low temperatures on mortality of larvae of *Trichoplusia ni* (Hbn.) fed on alfalfa sprigs dipped in suspension of polyhedrosis virus (1×10^8 polyhedra /ml.).

Temperature Group °C.	Total No. of Larvae[a]	Per Cent Mortality[b] (Days After Treatment)								
		0	6	9	12	15	19	27	42	45
15.5	50	0	2	10	40	78	100			
10.0	50	0	0	2	4	8	30	67	98	100

[a] Plus 50 control larvae per group.
[b] Cumulative (corrected by Abbotts' formula).

Field Tests.—Very limited field tests were undertaken in the fall of 1953. The first replicated field trial was set up to determine whether the polyhedrosis virus, when sprayed on lettuce plants, could cause mortality to the cabbage-looper population. This test was conducted during a period of warm weather in October and November (average high, 30° C.; average low, 14° C.; overall average, 22° C.) in an experimental plot of Great Lakes-59 lettuce at the University of California Imperial Valley Field Station at Meloland. The single-nozzle overhead application by hand sprayer of virus suspensions of concentrations of 600 thousand, 400 thousand, and 100 thousand polyhedra per ml., at the high rate of about 150 gallons per acre to lettuce plants the day after thinning, resulted in the death of many of the larvae in the test plots in from 6 to 9 days. Larval counts made on representative plants in the test and control plots were rather variable because of the continuous development of young larvae and the appearance of some virus-caused mortality in the control plots.

A second field test was set up in November, 1953, in a field of half-grown head lettuce near Heber in the Imperial Valley. The larval population was rather uniform as to size and appeared to have passed its peak at the ending of egg deposition. This test, conducted during a period of rather cool weather (average high, 24° C.; average low, 6.5° C.; overall average, 15.8° C.), involved single-nozzle overhead application by hand sprayer of a virus suspension of a concentration of 300 thousand polyhedra per ml. at rates of 75 and 37.5 gallons per acre to replicated plots, each consisting of a 100-foot long double row of lettuce. Replicated untreated controls were maintained.

Virus-killed larvae were evident in the test plots 25 days after application of the infective material, and a reduction of about 80% in insect population was noted. No signs of virus infections were observed in larvae in the control plots. Casual observation indicated that the test plots suffered less looper damage than the untreated control plots.

In November, 1954, a test was set up in a field of half-grown head lettuce of mixed varieties at the Imperial Valley Field Station. This test involved application of virus material of three concentrations (10 million, 5 million, and 1 million polyhedra per ml.) to replicated plots, each consisting of a 60-foot-long double row of lettuce. Single-nozzle overhead application by hand sprayer was at the rate of about 12 gallons per acre. One series of plots received only one application of the virus, while a second series of plots received two applications spaced 2 weeks apart. Replicated untreated control plots were maintained.

Because of the number of plots involved in the test and the large size of the lettuce plants in the field, the number of plants sampled for the presence of larvae of *Trichoplusia ni* was limited to five per plot. Records were kept of the number of larvae that fell within the general categories of small (first and second instar), medium (third and fourth instar), and large (fifth instar). The last two groups are considered to cause damage to the lettuce head;

the small larvae usually feed on the outer leaves.

Results of the sampling by larval counts of five randomly selected lettuce plants per replicate (fig. 1) indicate that high dosages of the virus are more effective than low dosages in reducing the level of the looper population, causing almost 100% mortality in the population of damaging larvae in the test plots. Indications are, also, that two applications of a single concentration are more effective than one, and are at least as effective as a single application of a more-concentrated suspension of the virus.

The effectiveness of the polyhedrosis virus in reducing the overall looper population was slightly veiled by the fact that application with a single overhead nozzle fails to place the infective material on the lower sides of the outer leaves, where early-instar larvae do their feeding. In lettuce fields where looper populations are on the increase, as in the test plots of 1954, overhead application permits the continued development of the young larvae for a period of about 2 weeks on the virus-free lower leaves before they start to feed on the top leaves that were contacted by the virus suspension. This delay plus the normal incubation period of the virus, which varies with the temperature, may result in considerable damage to the lettuce plants before the diseased loopers cease feeding and succumb. The problem may be overcome by adjustments in application technique. The use of multiple nozzles for better side coverage of the plants, for example, should result in increased mortality of the young larvae before they are large enough to cause serious damage to the lettuce heads.

SUMMARY.—Results of laboratory infectivity tests with the polyhedrosis virus on lettuce indicate that at moderate temperatures (about 22° C.) small larvae of the cabbage looper, *Trichoplusia ni* (Hbn.) succumb to the virus more quickly than large larvae, although mortality is about the same in both age groups after about 9 days. Treatments with less-concentrated virus suspensions extend the incubation period of the disease. This was especially noticeable in the group of older larvae, where mortality was quite low until after the seventh day following infection.

Temperature plays an important role in controlling the activity of the virus. Whereas 100% mortality was noted on the ninth day at a temperature of about 22° C., a temperature reduction of 6.5 degrees centigrade more than doubled the time required for complete kill. A further reduction of 5.5 degrees centigrade resulted in more than a five-fold increase in the time required for 100% larval death.

The first field trial in 1953, involving single-nozzle overhead application by hand sprayer of virus suspensions to newly thinned lettuce plants in the Imperial Valley during a period of warm weather, resulted in the death of many larvae in the test plots in from 6 to 9 days. A second test in 1953, on half-grown lettuce during a period of cool weather, resulted in high larval mortality on the 25th day after application.

In November, 1954, during a period of cool weather, virus concentrations of 10 million, 5 million, and 1 million polyhedra per ml. were applied by hand sprayer at the rate of 12 gallons per acre to half-grown lettuce in the Imperial Valley. One series of plots received one application, while a second series received two applications spaced 2 weeks apart. The results in terms of percentage of plants infested with damaging larvae indicate that high dosages of the virus are more effective than low dosages in reducing the level of the looper population. Two applications of a single concentration are more effective than one, and are at least as effective as a single application of a higher concentration of the virus. In terms of microbial kill of the insect, applications of the higher dosages of the virus resulted in almost 100% mortality to the damaging larvae in the test plots.

The field trials to date have been made with single-nozzle handsprayer equipment directly over the row, with the result that little infective material contacted the lower sides of the outer leaves where the eggs and newly hatched larvae are located. Future trials will involve the use of side nozzles for better coverage of the plants and should result in increased mortality to the younger larvae before they are large enough to cause serious damage to the lettuce heads.

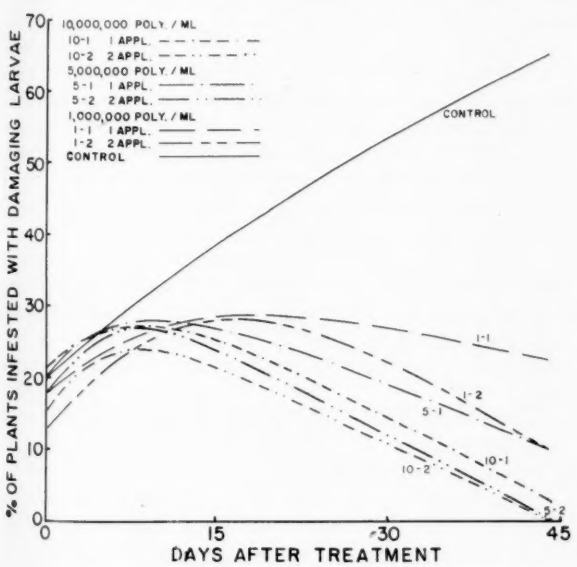

FIG. 1.—Effect of one and two applications of various concentrations (polyhedra per milliliter) of the polyhedrosis virus at low gallonage per acre on infestations of *Trichoplusia ni* (Hbn.), on lettuce.

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The Ratio of the Width to the Length of the Second Abdominal Sternite of the House Fly[1]

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Bigelow & LeRoux (1954) reported distinct morphological differences between certain DDT-resistant and DDT-nonresistant strains of house fly (*Musca domestica* L.) adults. One of the characters best suited to distinguishing resistant from nonresistant strains was the ratio of the width of the second abdominal sternite to the length of this same sclerite, a value designated by the authors as X_5. These investigators studied nine strains of flies. The susceptible Macdonald strain and the resistant Beltsville strain were known from laboratory tests, reported later by LeRoux & Morrison (1954), to be relatively susceptible and resistant at the time of measuring. Two other strains were considered as resistant.

Sokal & Hunter (1955) reported a study of a number of morphological characters in nine different house fly strains. Five of the strains used had been reported to be resistant by other workers. All strains were reared continually by the writers. Each generation of two of these strains was exposed to DDT. Tested by the writers by means of topical application of DDT in carbon tetrachloride, three strains were designated by them as actually resistant. They found the strains to differ in certain morphological characters but the same differences did not occur in successive tests. Characters correlated with resistance in one series of tests were not so correlated in others. There was marked variation in the characters measured when samples were drawn from different culture jars or different generations. Bigelow & LeRoux's X_5 value was less affected by specimen size than certain other characters, but not consistently related to either nominal or measured resistance. The authors concluded that none of the characters investigated was correlated with resistance and that such correlations were unlikely to be found since resistance appears to result from various causes.

This paper is an attempt to further assess the variations in Bigelow & LeRoux's value X_5 and to determine whether or not its value is in any way correlated with resistance.

MATERIALS, METHODS AND PROCEDURE.—Collections of flies preserved in 70% alcohol were obtained from 20 different laboratory strains. All collections were composite, each being made up of at least five and usually 10 samples drawn on successive weeks from laboratory cultures of the same strain. This procedure was aimed at randomizing any variations due to different nutritional conditions in different culture jars, to a culture being derived from a single clutch of eggs, or to changes from one generation to another. The successive samples were kept separate and flies for measurement drawn equally from each such sample. Most of the strains were supplied by workers in various testing laboratories throughout the United States and Canada in response to a request from W. S. McLeod, Pesticide Testing Laboratory, Department of Agriculture, Ottawa, Canada. The Wilson strain was measured from pinned male adults retained after the earlier work of Bigelow & LeRoux.

The different strains are designated by the strain name supplied by the sender and the name of the laboratory which sent the collection. In most instances the strain name suggests the presence or absence of resistance. No attempt was made to rear the strains involved at Macdonald or to test their resistance there.

Measurements were made with a Zeiss Filar Micrometer Eyepiece fitted by means of an adapter into one tube of a Zeiss Opton Stereo-microscope. All measurements were made at a magnification of $37\frac{1}{2}\times$. Each dimension was measured twice, once by moving the paired hair-lines from left to right and once by moving them from right to left. When the paired measurements varied more than 0.05 units (approximately 5%) they were discarded and both measurements made again. Measurements showing the desired agreement were recorded and averaged. The ratio of width to length was then determinated. Twenty-five specimens of each sex were measured for each strain.

All measurements were made by one or two workers, Miss J. Gustafson or the writer. Males only of the Wilson strain were measured from a sample from the same collection used by Bigelow & LeRoux. The males of the Mayo strain and females of the Ottawa Laboratory strain were measured by both workers using different samples from the same collections. The Macdonald strain, which has been reared continuously over the years at Macdonald College without any obvious change in its susceptibility level, was resampled and remeasured.

Separate analyses of variance were carried out on the data from male and female specimens and a combined analysis on the 19 strains for which both males and females were tested.

RESULTS.—Table 1 lists the source of each composite collection, its strain name, and the average X_5 ratio for males and females based on 25 specimens of each sex. The necessary differences between means are shown based on separate analyses of variance carried out on the samples of each sex and on an analysis of the 38 samples on which data are available for both sexes. The overall average for the sexes differed significantly. In seven of the 19 strains measured the means for the two sexes differed. Taken in all possible combinations of pairs of strains 95 out of 210 pairs of averages for the males show differences equal to or beyond that expected on a chance probability of $P=0.01$ while another 40 exceed the necessary differences for $P=0.05$. For the females the corresponding figures are 74 and 9 pairs, respectively, out of 190.

Table 2 sets forth the measurements from the same strains measured by different workers from collections taken at different times.

DISCUSSION.—The value of X_5 (table 1) appears to vary continuously over a wide range. The DDT '45 and DMC 5x strains yielded remarkably high values in both

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sexes, values comparable with the ones found for the Beltsville strain obtained from London, Ontario, by Bigelow & LeRoux (1954) and the OL strain of Sokal & Hunter (1955). These were all highly resistant strains. None of the strains showing very low ratios, with the possible exception of Wilson, have any known history of resistance.

The NAIDM (National Association of Insecticide and Disinfectant Manufacturers) '48 CSMA (Chemical Specialties Manufacturers Association) '51 strains show in several instances ratios in between the known susceptible Macdonald and the highly resistant strains. The ratios of some cultures of these strains considerably exceed those given by Bigelow & LeRoux for the presumably resistant Belleflower and Super Polard strains. It is noteworthy, however, that with the exception of the NAIDM '48 from Beltsville, the males in this group have a uniformity suggesting their common origin.

In no instance did different workers arrive at significantly different figures when measuring different samples of the same strain. This is true even where the specimens (Wilson) were pinned ones and the measurements made 2 years later, *e.g.*, Bigelow & LeRoux report an X_5 of 0.906 for a sample (mixed sexes) of the Wilson strain. A second sample of these pinned flies (males) measured by Morrison yielded a value of 0.95.

The Macdonald strain, resampled after 3 years of continuous culture showed little change in ratio (table 2). The Mayo strain, however, sampled somewhat less than a year later was just significantly different. If the variance due to different workers could be removed here the picture might be changed. It should be remembered, too, that the 1953 sample was a single and not a composite one. There is no evidence of reversal such as was found by Sokal & Hunter (1955) for some characters in their work.

Table 2.—X_5 values as influenced by continued breeding.

Strain	Year Measured	Workers	X_5 Males	X_5 Females
Macdonald	1956	Morrison	0.97	0.98
Macdonald	1953	Bigelow & LeRoux	0.925[a]	
Mayo	1953	Gustafson	1.02	1.03
Mayo	1954	Bigelow & LeRoux	0.95[a]	

[a] Mixed sexes.

Differences between the ratios for the two sexes of the same strains were (table 1) barely significant at the P= 0.01 point. This significance appears due to fairly large differences for seven of the 19 strains involved. The fact that this difference was much more marked in some strains than in others was also noted by Sokal & Hunter. These sex differences result in some changes in the order of placement if the measurement for the female sex is used in table 1. The same strains, however, would remain at the top and bottom of the list.

Resistance is not something an insect either has or hasn't. It is held by each insect in its own degree. Therefore, we cannot be certain of a real correlation between the value X_5 and resistance without having comparable measurements of the degree of resistance in each strain.

Table 1.—X_5[a] Ratios in different house fly strains.

Strain	Source	Measured by	Average X_5 for Samples of 25 Males	Average X_5 for Samples of 25 Females	Susceptibility
Wilson	Rutgers University, New Brunswick, N. J.	M	0.95[b]		S
Macdonald	Macdonald College, P. Que.	M	0.97	0.98	S
Pollard	Shell Development Co., Denver, Colo.	M	0.98	1.04	S
Normal Shell	Shell Development Co., Denver, Colo.	G	0.98	1.00	S
Mayo	Govt. Insecticide Testing Lab., Ottawa	M	1.00	—	S
NF (Babers)	Ent. Division, U.S.D.A., Washington, D. C.	G	1.00	1.04	S
C.S.	California Spray Chemical Co., Richmond, Calif.	M	1.01	1.07[d]	S
Ottawa Lab.	Govt. Insecticide Testing Lab., Ottawa	G	1.01	1.10[d]	S
Ottawa Lab.	Govt. Insecticide Testing Lab., Ottawa	M	—	1.06	S
NAIDM '48	Ent. Division, U.S.D.A., Beltsville, Md.	G	1.01	1.08[d]	S
Fisher	Science Service Lab., London, Ont.	G	1.02	1.02	R
Mayo	Govt. Insecticide Testing Lab., Ottawa	G	1.02	1.03	S
DDT Shell	Shell Development Co., Denver, Colo.	M	1.03	1.05	R
R (Babers)	Ent. Division, U.S.D.A., Beltsville, Md.	M	1.03	1.01	R
NAIDM '48	Communicable Disease Center, Atlanta, Ga.	G	1.07	1.15[d]	S
CSMA '51	Ent. Division, U.S.D.A., Washington, D. C.	G	1.07	1.08	S
497	Communicable Disease Center, Atlanta, Ga.	G	1.08	1.06	R?
CSMA '51	Standard Oil, Whiting, Ind.	G	1.08	1.11	S
Roberds	Communicable Disease Center, Atlanta, Ga.	G	1.09	1.08	R
CSMA '51	Carbide & Carbon Chemicals, Boyce Thompson Institute, Yonkers, N. Y.	M	1.10	1.03[d]	S
DDT '45	Communicable Disease Center, Atlanta, Ga.	G	1.19	1.20[d]	R
DMC5x	Communicable Disease Center, Atlanta, Ga.	G	1.27	1.20[d]	R
		Average	1.05[c]	1.07[c]	

[a] X_5=ratio of width to length of second abdominal sternite.

[b] *Necessary differences:*

	For: P=0.05	P=0.01
Between strain means (males)	0.05	0.07
Between strain means (females)	0.06	0.07
[c] Between averages for sexes	0.01	0.015

[d] Sexes differed significantly.

Even if such figures were available, resistance would undoubtedly vary continuously through a series of strains so that some pairs would not be separable by known techniques. Further evidence for or against the postulated correlation could be derived from measurements on cross bred strains. Until such work is done, the figures presented here show a reasonable genetic constancy in the value X_5 and point to at least a partial correlation with resistance. This seems sufficient to warrant a further search for morphological correlates with resistance in this and other species of insects.

SUMMARY.—Measurements were made of the ratio of the width to the length of the second abdominal sternite of adult house flies of both sexes from 19 different laboratory cultures being reared in a number of widely separated insecticide testing laboratories. This ratio, designated as X_5, varied from culture to culture, but in general large ratios were associated with cultures said to be DDT-resistant and small ratios with cultures said to be susceptible, but the correlation was not perfect. Variation due to different workers measuring samples of the same culture were not significant. Variation between samples drawn from the same cultures at long time intervals were significant in one instance but not in another. Cultures with a known common origin in the past showed ratios with a narrow range. The ratios differed for the different sexes of the same strain in seven out of 19 instances.

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Further Studies on Resistance of Human Body Lice to Insecticides[1]

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Eddy *et al.* (1955) found that laboratory colonies of the body louse, *Pediculus humanus humanus* L., developed a high degree of resistance to DDT and slight resistance to lindane, lauseto neu, and pyrethrins after continuous exposure to cloth treated with these insecticides. This paper discusses the continuation of those studies.

DDT.—A strain of DDT-resistant lice from Korea developed extreme resistance in 15 generations when maintained on cloth treated with a 0.01% DDT solution. This colony (DDT colony A) has been maintained in the same manner for 70 generations. The susceptibility of these lice to DDT and 11 of its analogs was determined by the beaker test method. Circular pieces of wool cloth were saturated with acetone solutions of the compounds and, after having been dried on pins, each was placed in the bottom of a 50-ml. glass beaker. Then 20 young adult lice were placed on the cloth. The beakers were held at a constant temperature of 80° F. and 60 to 70% relative humidity, and the mortality was recorded after 24 hours. Moribund lice were included with dead lice in computing the per cent mortality.

The results are presented in table 1. The F_{70} generation was more resistant than normal lice to five DDT analogs as well as to DDT. Lice of colony A were not resistant to four other analogs. Dianisyl neopentane and MR-60 (chlorobis(*p*-chlorophenyl)-methane) were not effective against either strain.

A subcolony (DDT colony B), separated from colony A in the 17th generation on treated cloth and reared thereafter on untreated cloth, had lost some resistance after 15 generations (Eddy *et al.* 1955). Continued maintenance on untreated cloth resulted in no further loss of resistance through the 45th generations, when colony B was discontinued. The general trend of resistance is shown in the results of beaker tests which were conducted with lice of each generation (table 2). Although concentrations as low as 0.1% killed almost half the lice, complete mortality was rarely obtained even with 10%.

Dimite (DMC) and MR-60, which increase the effectiveness of DDT against resistant house flies, were tested in combination with DDT against lice of the extremely resistant DDT colony A, the moderately resistant DDT colony B, and the regular colony that had been maintained in the laboratory for 14 years without exposure to insecticides. Beaker tests were conducted with various concentrations of DDT, alone and combined with the synergists at a ratio of 5:1. The LC-50's are presented in table 3. The resistance of colony A was so high that no mortality occurred in the usual 24-hour exposure, as the lice were entirely immune to concentrations as high as 10%, both with and without the synergist. In other tests none were killed by a 48-hour exposure to technical DDT. The synergists caused a slight but not significant increase in effectiveness against colony B and none against the regular colony. Colony B was approximately 50 times as resistant to DDT as the regular colony.

Eddy *et al.* (1955) reported a high degree of resistance in a strain of lice separated from the regular colony and reared on cloth treated with acetone containing 0.001%

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[2] G. W. Eddy was in charge of the project when some of these studies were started. D. L. VanNatta assisted in performing some of the tests and P. N. Mason and R. L. Bonner in rearing colonies.

Table 1.—Susceptibility of DDT-resistant (DDT colony A) and nonresistant (regular colony) body lice to DDT and 11 of its analogs. (3 replications.)

Insecticide	Per Cent Concentration	Per Cent Mortality After 24 Hours	
		Colony A	Regular Colony
Ethane, 2,2-bis(*p*-bromophenyl)-1,1,1-trichloro-	10	3	85
Ethane, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)- (*TDE*)	10	3	80
Ethane, 1,1,1-trichloro-2,2-bis-(*p*-chlorophenyl)- (*DDT*)	10	7	97
Ethane, 1,1,1-trichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)-	10	0	22
Ethylene, 1,1-bis(*p*-bromophenyl)-2,2-dichloro-	10	3	28
Methane, chlorobis(*p*-chlorophenyl)- (*MR-60*)	10	0	10
Propane, 1,1-bis(*p*-chlorophenyl)-2-nitro- (*Prolan*)	10	0	97
Propane, 1,1-(*p*-methoxyphenyl)-2,2-dimethyl- (*dianisyl neopentane*)	10	0	3
Ethane, 1,1-bis(chlorophenyl)-	5	98	95
	2.5	82	90
	1	68	83
	.5	53	80
	.25	43	83
	.1	35	68
Ethane, 1,1,1-trichloro-2,2-bis(*p*-fluorophenyl)- (*DFDT*)	.1	100	—
	.05	100	—
	.025	93	100
	.01	17	83
	.005	—	17
Ethane, 1,1,1-trichloro-2,2-bis-(*p*-methoxyphenyl)- (*methoxychlor*)	2.5	82	—
	1	35	97
	.5	43	95
	.25	3	77
	.1	3	52
Methane, butoxy(*alpha*-trichloromethylbenzyloxy)-	1	93	93
	.5	43	38
	.25	12	7
Untreated check	—	5	0

of DDT. This concentration gave a low selection rate, as the lice did not live as long or lay as many eggs as those on untreated cloth. DDT colony D was later separated from the regular colony and maintained on cloth treated with a 0.0001% DDT-acetone solution to determine whether resistance could be developed by exposure to a dosage of DDT too low to cause any selection. The mortalities among representative lots of lice from hatching to oviposition are given in table 4. The average mortality for seven generations was lower for lice of colony D maintained on DDT-treated cloth than for the regular colony on untreated cloth.

Table 2.—Per cent mortality of successive generations of Korean body lice of DDT colony B and the regular colony treated with different concentrations of DDT.

Colony and Generation	Per Cent Concentration of DDT									
	10	5	2.5	1.0	0.5	0.25	0.1	0.05	0.025	0.01
DDT:										
Parent	15	—	—	5	—	—	0	—	—	5
F_1	0	25	5	15	—	—	—	—	—	—
F_3	20	10	10	25	—	—	—	—	—	—
F_6	25	35	15	10	—	—	—	—	—	—
F_7	35	45	35	40	—	—	—	—	—	—
F_9	75	75	70	75	65	65	45	35	30	40
F_{11}	68	70	78	68	80	78	63	48	33	33
F_{13}	75	85	75	65	75	65	45	45	25	45
F_{24}	45	45	35	50	35	35	20	5	10	
F_{25}	65	75	65	60	65	60	30	15	35	0
F_{26}	80	78	68	67	62	55	47	42	25	15
Regular[a]	—	—	—	—	—	—	—	—	99	92[b]

[a] Average of 10 generations.
[b] 73% mortality at 0.005%, 28% at 0.0025%, and 7% at 0.001%.

Table 3.—LC-50 of three strains of body lice treated with DDT alone and with synergists.

Synergist	Colony A	Colony B	Regular Colony
None	>10.0	0.13	0.0027
Dimite	>10.0	.08	.0027
MR-60	>10.0	.05	.0024

Table 4.—Per cent mortality in various stages of lice from DDT colony D, maintained on treated cloth, and the regular colony on untreated cloth.[a]

Colony and Generation	Nymphs (Instar)			Adults		All Stages
	First	Second	Third	During Preoviposition Period	During First 5 Days of Oviposition Period	
DDT, parent	2.8	5.8	2.8	0.8	2.8	15.0
Regular	2.8	4.8	6.5	7.5	12.0	33.6
DDT, F_1	10.3	7.3	2.3	1.3	4.0	25.2
Regular	0	13.0	6.8	3.0	2.8	25.6
DDT, F_2	9.8	14.3	10.0	4.5	11.5	50.1
Regular	3.0	10.8	8.0	4.0	16.8	42.6
DDT, F_3	9.3	13.8	2.8	1.5	7.3	34.7
Regular	7.0	9.3	7.0	4.0	11.5	38.8
DDT, F_4	8.0	2.3	1.8	.5	7.8	20.4
Regular	5.3	11.3	14.5	2.5	13.8	47.4
DDT, F_5	5.0	3.8	1.8	.3	4.8	15.7
Regular	17.5	7.5	9.5	2.5	7.8	44.8
DDT, F_6	7.5	1.8	.8	0	2.5	12.6
Regular	3.3	7.0	3.8	2.8	3.0	19.9
Average all generations						
DDT	7.5	7.0	3.2	1.3	5.8	24.8
Regular	5.6	9.1	8.0	3.8	9.7	36.2

[a] Average of 2 lots of 200 newly hatched nymphs each.

Results of beaker tests against successive generations of this colony are shown in table 5. A slight resistance to DDT developed by the 12th generation, but there was no additional increase after 26 generations and the colony was therefore discontinued.

LINDANE, LAUSETO NEU, AND PYRETHRINS.—Eddy *et al.* (1955) found that lice maintained for five generations on cloth treated with low concentrations of lindane and lauseto neu, or for 17 generations with pyrethrins, developed a slight resistance to these insecticides. No

Table 5.—Per cent mortality of body lice from DDT colony D and the regular colony treated with different concentrations of DDT.

Colony and Generation	Per Cent Concentration of DDT						
	0.1	0.05	0.025	0.01	0.005	0.0025	0.001
DDT:							
Parent	—	—	—	85	60	0	5
F_1	—	—	—	90	55	8	5
F_2	—	—	95	95	40	5	0
F_3	—	—	85	90	25	0	0
F_4	—	100	100	95	90	20	0
F_8	—	100	95	90	75	15	0
F_{12}	100	95	90	70	15	0	0
F_{16}	90	95	80	85	5	5	5
F_{21}	98	90	85	70	20	15	13
F_{26}	92	93	93	68	23	5	7
Regular[a]	—	—	99	89	66	23	7

[a] Average of 10 generations.

Table 6.—Susceptibility to lindane of body lice from colonies reared on treated cloth.

Colony	Rearing Concentration (Per Cent)	Generation	Per Cent Mortality at Indicated Concentration			
			0.0025	0.001	0.0005	0.00025
Parent	—	P	100	100	68	33
A	0.00005	F_2	—	70	55	20
		F_4	100	73	23	3
		F_8	100	63	35	0
		F_{12}	100	95	35	5
		F_{16}	100	100	80	20
		F_{24}	100	100	95	35
		F_{30}	100	90	35	0
		F_{34}	95	45	0	0
B	.0001	F_1	100	45	0	0
		F_3	100	100	80	15
		F_6	100	100	75	5
C	.00025	F_1	100	100	35	0
		F_3	100	80	20	0
		F_6	100	85	25	0
D	.0005	F_1	100	60	0	0
E	.001[a]	F_1	95	53	5	3
Regular[b]	—	—	100	99	86	35

[a] High mortality in colony at this concentration.
[b] Average of 10 generations.

resistance to pyrethrins plus sulfoxide was developed.

Maintenance on lindane, lauseto neu, and activated pyrethrins was continued for 30 to 35 generations without additional increase in resistance. The concentration of the insecticides was then progressively increased to provide selection in the colonies. The increases were made first at intervals of six generations and later at one generation. Results of beaker tests in successive generations showing the susceptibility of these colonies to the respective insecticides are given in tables 6, 7, and 8. Each developed about twofold resistance at the lowest concentration, but progressively increasing the concentration failed to increase the resistance. All colonies were killed as a result of exposure to the highest concentrations.

STROBANE.—A strain of lice was separated from the regular colony and maintained for 13 generations on cloth

Table 7.—Susceptibility to lauseto neu of body lice from colonies reared on treated cloth.

Colony	Rearing Concentration (Per Cent)	Generation	Per Cent Mortality at Indicated Concentration					
			0.5	0.25	0.1	0.05	0.025	0.01
A	0.0025	F_2	—	—	100	95	68	18
		F_6	—	100	95	15	5	8
		F_8	—	100	95	50	3	—
		F_{12}	—	100	85	45	0	—
		F_{18}	—	100	100	80	85	0
		F_{24}	—	100	95	40	5	0
		F_{30}	—	95	75	15	0	0
		F_{32}	—	95	80	10	5	5
B	.005	F_1	90	100	100	90	50	10
		F_3	100	100	100	100	70	0
		F_6	90	90	90	85	45	0
C	.01	F_1	95	95	90	55	15	0
		F_3	85	100	100	30	0	5
		F_6	95	95	60	5	0	5
D	.025	F_1	95	95	90	10	5	0
E	.1[a]	F_1	95	93	52	5	0	0
		F_3	100	95	100	80	0	0
Regular[b]	—	—	100	100	100	89	37	14

[a] High mortality in colony at this concentration.
[b] Average of 10 generations.

Table 8.—Susceptibility to activated pyrethrins of body lice from colonies reared on cloth treated with low dosages of activated pyrethrins.[a]

Colony	Rearing Concentration (Per Cent)	Generation	Per Cent Mortality at Indicated Concentration of Pyrethrins				
			0.01	0.005	0.0025	0.001	0.0005
Parent	—	P	100	100	100	25	10
A	0.0001	F_1	—	100	90	0	0
		F_4	—	100	100	20	0
		F_8	—	100	100	0	5
		F_{12}	—	100	100	30	5
		F_{18}	—	100	100	5	0
		F_{24}	—	95	50	0	0
		F_{30}	—	100	40	10	0
B	.00025	F_1	—	100	95	25	10
		F_3	—	100	100	65	5
		F_6	—	100	100	10	5
C	.0005	F_1	—	100	95	20	0
		F_3	—	100	75	15	0
		F_6	—	100	40	0	0
D	.001[b]	F_1	—	100	70	0	0
		F_3	100	100	60	30	40
		F_4	75	55	22	0	0
Regular[c]	—	—	—	100	99	30	11

[a] Sulfoxide used with pyrethrins at a ratio of 10 to 1.
[b] High mortality in colony at this concentration.
[c] Average of 10 generations.

treated with acetone containing 0.0001% of Strobane. Slightly less than twofold resistance was developed.

DISCUSSION.—March & Metcalf (1950) and Hoskins & Gordon (1956) point out that DDT-resistant house flies show normal susceptibility to Prolan and Dilan, which cannot be dehydrochlorinated. This they offer in support of the theory that dehydrochlorination is the major mechanism of DDT-resistance in house flies. However, as shown in table 1, DDT-resistant lice are also resistant to Prolan. Busvine (1953) shows that DDT-resistant flies and lice are also resistant to dianisyl neopentane, which also cannot be dehydrochlorinated because it contains no chlorine. In the tests reported in table 1, neither DDT-resistant nor regular lice were susceptible to this material when exposed to cloth impregnated with a 10% solution in acetone. Hoskins & Gordon (1956) include as further evidence of the importance of dehydrochlorination in resistance the fact that DDT synergists such as Dimite (DMC) increase the effectiveness of DDT by blocking dehydrochlorinase. Neither Dimite nor MR-60 increased the effectiveness of DDT significantly in tests against body lice (table 3). This evidence suggests that dehydrochlorination is not the important mechanism of DDT-resistance in body lice.

Perry & Buckner (1955) found that lice from both the DDT colony A and the regular colony absorbed very little DDT.

SUMMARY.—Laboratory studies were conducted on the development and nature of resistance to various insecticides in the body louse, *Pediculus humanus humanus* L. A strain of highly DDT-resistant body lice (colony A) was more resistant than normal lice to five DDT analogs but not to four others. A strain separated from colony A and reared on untreated cloth lost some resistance in 15 generations, but no more by the 45th generation.

Dimite (DMC) and MR-60 (chlorobis(*p*-chlorophenyl) methane) failed to increase the effectiveness of DDT against a highly resistant, a moderately resistant, and a nonresistant strain of lice.

A colony of lice maintained for 26 generations on cloth treated with 0.0001% DDT solution failed to develop more than twofold resistance. Mortality in the colony did not exceed that on untreated cloth.

Colonies of lice reared for 13 to 35 generations on cloth treated with low concentrations of lindane, lauseto neu, Strobane, and pyrethrins plus sulfoxide developed less than twofold resistance to these insecticides.

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Effects of Some Insecticidal Materials on Larval Populations of the Alfalfa Weevil in Southern Pennsylvania[1]

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The alfalfa weevil, *Hypera postica* (Gyll.), was first reported from Pennsylvania in the spring of 1952 when it was found in York and Lancaster counties near the Maryland border. Since that time it has spread rapidly throughout the southeastern section of the State. At present, it is known to occur in 25 counties south of a diagonal drawn roughly across the State from the New York border on the Delaware River southwest to Fulton County. Although the highest population levels still probably occur in the general area where it was first found, this insect is rapidly becoming a serious problem in many counties of southeastern Pennsylvania. The available evidence indicates that it will eventually become established in all parts of the State where alfalfa is grown.

In fields where the weevil is well established and abundant, the first crop of alfalfa may be almost completely defoliated resulting in a loss of approximately two-thirds of its nutritive value. Partly grown larvae remaining in the field after the first harvest and possibly newly emerged adults may retard the second crop or, when unusually abundant, may kill the plants entirely. Thus, considerable damage to the second crop may also occur.

Data on the life history and seasonal-behavior patterns of the alfalfa weevil in the East are far from complete. Various limited aspects of its biology and control have been investigated in Maryland by Poos & Bissel (1953) and App (1954), and in Delaware by Milliron & MacCreary (1955) and Milliron (1956). These workers have shown that the eggs, especially those deposited near the onset of cold weather, may survive winter temperatures and hatch the following spring. Possible winter survival of the larval stage also is indicated. It seems quite probable, however, that the largest segment of the larval population for a given season develops from eggs deposited in the spring by overwintered females.

It has been demonstrated (App 1954, Milliron & MacCreary 1955) that both heptachlor and dieldrin are effective in the control of the larval stages of this insect. However, a single early-season application, with the formulations and rates currently used, is not sufficient to suppress the larval populations during the entire period of development of the first crop of alfalfa. This means that (1) either the initial application must be delayed until considerable foliage injury has occurred, or (2) two applications must be employed. These must be timed to provide effective plant protection for the duration of the development of the first crop. A zero residue tolerance for dieldrin on forage crops at the present time eliminates it from consideration in either case.

In recent preliminary experiments, Muka (1957) has shown that granulated heptachlor, applied early in the spring at the rate of 1.5 pounds of actual toxicant per acre, provided an effective control of the alfalfa weevil under Virginia conditions.

The experiments described in this paper were designed to provide information on the effects of certain insecticidal environments on the larval-population patterns of the alfalfa weevil existing in southern Pennsylvania during the spring of 1956. The work was conducted in a 7-year-old planting of alfalfa in the eastern section of Adams County and covered the interval between the early growth stages of the plants and the harvest of the first crop. The alfalfa had been severely damaged by the weevil during the preceding season, and no control measures had been employed either against it or the meadow spittlebug, *Philaenus leucophthalmus* (L.).

MATERIALS AND METHODS.—All treatments were replicated four times in randomized blocks using plots approximately 1/80 acre in size (30 ft.×18+ft.). Materials used included endrin, dieldrin, lindane, heptachlor, and a 1:1 heptachlor-BHC combination as emulsifiable concentrates. Heptachlor was also used in granulated form. The emulsifiable concentrates were applied at the rate of 100 gallons of water per acre using a wheelbarrow-

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type Myers sprayer operated at a pressure of approximately 225 p.s.i. The single treatment of granulated heptachlor was broadcast by hand.

Larvae were first observed in the field on May 4 when an average of 5 per 100 sweeps of a 12-inch net was recorded. Noticeable foliage injury had occurred on May 11, and population levels increased thereafter to maximum numbers on or slightly before June 5.

Net sampling was employed to determine the relative abundance of larvae in the experimental plots. All samples were taken by the same individual and at approximately the same time of day on each occasion. Ten sweeps of a 12-inch net were taken from each plot on the dates indicated, using similar diagonals as sampling lines and traversing approximately the middle four-fifths of their length. A given diagonal was never used as a sampling line twice in succession.

In taking the first two series of samples (May 18 and 25), the larvae from each plot were placed in a large white pan, counted, and returned to the same general area in which they were captured. The two subsequent series of samples were placed in alcohol and counted later. No effort was made to estimate the relative abundance of adults. In net samples taken during the day, the adult weevil seldom appears in the samples in proportion to its abundance in the field.

RESULTS.—The data on treatments and larval-population patterns observed in the different environments compared with the population development in untreated areas are shown in table 1.

It will be noted that all treatments applied on April 27, when the alfalfa was slightly over 4 inches high and before there was noticeable larval activity, resulted in a significant depression of the population levels observed 3 weeks later. However, data taken the following week (May 25) showed a considerable population recovery in all plots except those treated with granulated heptachlor. The population levels observed on June 5 were similar to or had fluctuated only slightly from the levels obtained 11 days earlier.

The final records taken on June 14, when pupation was well underway and the larval population had begun to decline, revealed some interesting relationships between these data and the initial abundance observed in the different environments. Materials which had exhibited high initial effects in the early stages of population growth appeared to possess a similar depressing effect during its decline. This apparent influence is not thought to be due to any insecticidal factor operative at the time the samples were taken. Rather, it would appear to be more closely associated with the magnitude of the populations which were able to develop and survive in the different environments prior to this time.

Treatments applied on May 4 when the plants were 8 to 10 inches high coincided approximately with incipient larval activity. These treatments resulted in a depression of larval population levels quite similar to those observed for the same materials applied 1 week earlier. The effects were still noticeable 3 weeks later (May 25), but on June 5 recovery was quite evident. In two instances, the population levels were higher at this time than in plots treated with the same materials 1 week earlier. The apparent effects during the larval population decline were similar to those observed in the earlier treatments.

At the time the last two treatments were applied on May 11 and 18, foliage injury varied from moderate on the earlier date to quite severe 1 week later. On May 11 the alfalfa was approximately 15 to 18 inches high, while the height of many plants exceeded 21 inches on May 18. The use of tractor-drawn spray equipment in the field on either date would have caused considerable damage to the crop.

The initial effects on the larval population levels when heptachlor was applied on May 11 at the rate of 6 ounces per acre were similar to those observed on plots treated identically 1 week earlier. Also, it will be noted that these effects were approximately the same as those recorded when the same material was applied at the rate of 12 ounces per acre on April 27. The effects of the May 11 treatment remained evident until June 5 when population recovery was indicated. Foliage injury in these plots was noticeable at harvest, but was not particularly severe.

The final application of heptachlor on May 18 produced initial effects on the larval population similar to those observed for the same material in previous treatments. No population recovery was noted. This treatment, however, had little or no practical significance because substantial damage to the crop had already occurred.

Foliage injury at harvest ranged from moderate to severe in all plots except those treated with granulated heptachlor. This injury occurred in some plots because the materials were relatively ineffective in reducing the larval populations from the beginning, while in others the materials with high initial effectiveness did not maintain it throughout the entire period of larval activity. In plots where applications were purposely delayed until larval populations had developed to relatively high levels, the reason for the injury is obvious. Damage in plots where high population levels developed because of initial ineffectiveness of the materials was almost as severe as that in the untreated areas.

Table 1.—Population levels of alfalfa weevil larvae observed in plots receiving different insecticidal treatments during the spring of 1956 in Southern Pennsylvania.

MATERIALS[a]	APPLICATION RATE (OZ./A. ACTUAL)	AVERAGE NUMBER OF LARVAE[b] PER PLOT SAMPLE ON			
		May 18	May 25	June 5	June 14
Applied on April 27					
Endrin	4	22	83	101	56
Endrin	8	16	81	52	31
Dieldrin	4	11	45	50	5
Dieldrin	8	10	49	31	4
Heptachlor	6	5	40	50	8
Heptachlor	12	3	28	28	3
Heptachlor-BHC (g.i.)	3:3	8	33	47	13
Lindane	4	28	99	163	80
Heptachlor (2½%) (granules)	44	1	2	2	1
Applied on May 4					
Heptachlor	6	2	11	37	5
Heptachlor-BHC (g.i.)	3:3	4	28	99	7
Dieldrin	4	2	28	69	5
Applied on May 11					
Heptachlor	6	2	3	26	5
Applied on May 18					
Heptachlor	6	61[c]	1	2	3
Untreated		72	150	212	80

[a] Unless otherwise indicated, all formulation were emulsifiable concentrates: endrin, 1.6 lbs./gal.; dieldrin, 1.5 lbs./gal.; heptachlor, 2 lbs./gal.; BHC, 1 lb. gamma isomer/gal.; and lindane 1.6 lbs./gal.

[b] Rounded figures to nearest whole number; based on four replicates.

[c] Population levels observed prior to treatment on this date.

Although the data in this paper are based on larval populations, it is quite evident that we have, in some instances at least, indirectly measured the effects of the insecticides on the adult populations prior to and during spring oviposition. Preliminary data on the behavior of the adult weevil during the spring indicate that it spends considerable time on the ground and on or in the debris near it. In the case of plots treated early in the season with granulated heptachlor, it is evident that we have significantly reduced the adult population prior to spring oviposition, and have at the same time practically eliminated any segment of the larval population which may have developed from overwintered eggs.

The total number of weevil larvae occurring in all samples from the granulated heptachlor-treated plots is further evidence of its effectiveness in maintaining an environment unfavorable to the insect for the duration of the experiment. In the 16 ten-sweep samples taken during the 4-week period, only 19 larvae were observed. The appearance of the foliage taken 4 days prior to harvest compared with foliage from untreated plots is shown in figure 1.

The amount of granulated heptachlor employed in these experiments is probably somewhat above that required to provide adequate control of the alfalfa weevil. No data were obtained on the minimum amounts of this or other granulated materials necessary to provide plant protection nor on their emulsifiable concentrate or wettable powder equivalents.

The data presented in this paper demonstrate that the larval populations of the alfalfa weevil as they exist in southern Pennsylvania may be effectively suppressed,

FIG. 1.—Alfalfa foliage (left) from plots treated with granulated heptachlor compared with foliage (right) from untreated plots.

during some years at least, by the early-season application of a suitable insecticide formulation. Conclusive evidence was obtained with granulated heptachlor.

SUMMARY.—The larval population levels of the alfalfa weevil developing and surviving on alfalfa treated with various insecticidal materials were observed in southern Pennsylvania during the spring of 1956. Insecticides used in replicated plots of approximately 1/80 acre included endrin, dieldrin, heptachlor, lindane, and a 1:1 heptachlor-BHC combination as emulsifiable concentrates. Heptachlor was applied in granulated form at a higher rate of application. Nine insecticidal treatments were applied during the early growth stages of the alfalfa (Apr. 27), three at the approximate time of incipient larval activity (May 4), and two when larval populations had reached significant levels (May 11 and 18).

Net sampling was employed at four intervals to determine the relative magnitude of the larval populations which were able to develop in the different environments. All treatments applied prior to larval activity in the spring, as well as those applied near its incipience, produced significant initial reductions in larval populations. However, population recovery occurred in all plots except those treated in early spring with granulated heptachlor at the rate of 44 ounces (active) per acre and where the initial and subsequent effectiveness was sufficient to protect the plants until harvest of the first crop 53 days later. Of the two treatments of heptachlor, each at the rate of 6 ounces per acre applied as an emulsifiable concentrate when larval populations had reached significant levels, only the later one maintained its effectiveness until harvest of the first crop. The later treatment had little practical significance because severe foliage injury had occurred prior to application.

The data indicate that the larval populations of the alfalfa weevil in southern Pennsylvania may be effectively suppressed by early-season applications of insecticides when suitable materials and rates of application are employed.

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The Burrowing Nema Situation in Florida[1]

W. BIRCHFIELD[2]

Since Suit & DuCharme (1953) reported that the burrowing nema (nematode) *Radopholus similis* (Thorne) is the causal agent of a destructive citrus disease known in Florida as "spreading decline" much excitement and interest in this tiny animal has prevailed. Due to the economic importance of citrus in the livelihood of the people of Florida and of the nation nearly 2½ million dollars were appropriated for research and efforts toward control of this destructive pest.

The purpose of this paper is to present the general knowledge that has accumulated on the causal organism as well as the disease. Consequently, the taxonomy and description, life cycle and pathogenicity, host plants, geographical distribution and dissemination of the burrowing nema are reviewed, as well as the symptoms and economic importance of the disease. Comments are also made on the research and efforts toward the control of the disease.

THE ORGANISM.—*Taxonomy and description.*—The common name, burrowing nema, was given this organism because of its habit of forming burrows in the cortical parenchyma of its host plants. It was first recorded scientifically in 1893 by Cobb as *Tylenchus similis*. It was associated at that time with a root disease of banana, *Musa sapientum* Kuntze in the Fiji Islands but only the males were observed. Cobb (1915) next reported it from Hawaii, associated this time with sugarcane, *Saccharum officinarum* L. Since both males and females were found in abundance it was believed to be a new species and assigned the name *Tylenchus biformis*. It was next reported in 1915 from the island of Jamaica by S. F. Ashby. Cobb then realized the relationship of what he had originally called *T. similis* and later *T. biformis*. He therefore established *Tylenchus similis* as having priority and redescribed the organism.

Filipjev (1936) placed *Tylenchus similis* in the genus *Rotylenchus*. Goodey (1932) had previously synonymized *Tylenchus acutocaudatus* which Zimmerman described from coffee with *Anguillulina similis*. When Thorne erected the genus *Radopholus* in 1949 the burrowing nema was placed as the type species and became *Radopholus similis* (Cobb, 1893) Thorne, 1949.

The burrowing nema is an average-sized, plant-parasitic nema of endoparasitic habit. The females average about 0.66 mm. in length by about 0.26 mm. in width, while the males are somewhat smaller. Sexual dimorphism is distinct in that the head of the male is bulbular and offset, with a weak stylet and slight internal sclerotization of the head in contrast to opposite characters in the female.

Life cycle and pathogenicity.—The entire life cycle of the burrowing nema takes place within the invaded plant roots including copulation and egg deposition. They generally attack the cortical parenchyma of young, succulent roots slightly back of the tips, enter, and form burrows or

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small cavities. These cavities soon contain both males and females, as well as eggs and larvae in all stages. The lesions thus caused may or may not show discoloration depending on the host plant involved and the fungi and bacteria associated. The nemas may work themselves forward thereby furnishing avenues of infection for soil-borne fungi and bacteria but the central cylinder is left intact. In the case of citrus, root infection results in a decline manifested by small leaves and fruit, dieback, and wilting of leaves. Infected trees seldom die outright but lapse into a poor physiological state gradually ceasing to produce a profitable crop. A general belief has been expressed that this organism is responsible, at least in part, for a decline of avocado (DuCharme & Suit 1953).

Spreading decline of citrus differs from several additional declines primarily in the pattern and rapidity of spread. It moves in all directions from centers of infestation, uphill as well as downhill, at about 50 feet per year.

Symptoms of disease of ornamental plants infected with the burrowing nema are suspected in some cases but have not been demonstrated experimentally. However, in most cases lesions of various types may be found on the roots from which the organism may be dissected. As these areas dissolve, the nemas move into partly firm or stable tissues, thus lesions enlarge.

Dissemination.—Besides the natural spread from tree to tree, the burrowing nema is transmitted to new planting areas in the roots of citrus nursery stock. It is believed to transfer from ornamental plants to citrus around homesites. It is also carried on mechanical equipment and implements to some extent. Drainage and erosion water undoubtedly function to spread the organism (DuCharme 1955) and there may be some possibility of occasional dissemination in irrigation water from lakes and streams.

Host plants and geographical distribution.—Since the early reports of the burrowing nema in sugarcane, banana and coffee, Godfrey (1931) established nutgrass (*Cyperus rotundus* L.) as a host. Cassidy & van Zwalunwenberg (1927) found it in the pods of *Cajanus indicus* Spreng., in the roots of pineapple (*Ananus sativus* Schult.), in bulbs of the edible canna (*Canna edulis* Ker.), and in sweet potato (*Ipomoea batatas* Lam.). Menzel (1929) reported it from the roots of the tea plant (*Thea sinensis* L.) from Java. The first report of this organism in the United States was that of Rands (1929) in sugar cane from Louisiana. After the report of citrus as a host plant in 1953, Brooks (1954, 1955) and Birchfield (1956) published records of many weeds and ornamental hosts from Florida. Sher (1954) recently reported several host plants from Hawaii so that now the list of hosts of the burrowing nema numbers about 150 or more representing at least 30 widely different plant families.

Aside from the places of original descriptions the burrowing nema has also been found in California, Japan, Formosa, South India, Puerto Rico, the Dutch East Indies and Africa.

THE DISEASE.—*Economic Importance.*—At the present time the disease, spreading decline, is regarded as the most serious threat to the citrus industry in Florida since bacterial canker. It is known only in Florida where about 8,000 acres of groves are involved. If uncontrolled the disease will probably involve a considerable acreage in the next 10 years. The burrowing nema has been found in almost every county in the citrus area (fig. 1). Thousands of samples have been processed from many properties and infestations of varying intensity have been reported in about 25 counties. Most of the positive samples are from citrus groves but a high percentage are from ornamentals. Field sampling revealed that the burrowing nema was most commonly recovered from citrus growing in the high sandy ridge area and seldom from citrus growing in low, poorly-drained soils; however, it is often recovered from banana in poorly-drained soils. Most of the infestations were found in the heart of the citrus belt of Polk and Highlands counties.

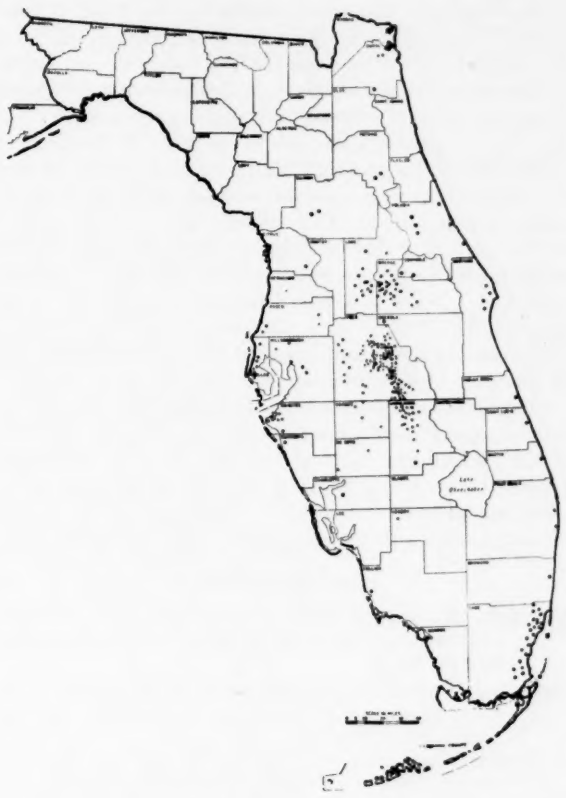

FIG. 1.—A map showing distribution (black dots) of the burrowing nema, *Radopholus similis*, in Florida.

Research and Control.—Research on the burrowing nema and spreading decline is currently conducted by the State Plant Board, University of Florida, and the Nematology and Fruit and Nut Crop Sections of the United States Department of Agriculture. An effective and successful method of hot-water treatment of citrus-nursery stock has been established by Birchfield (1954) in the industry to assure the grower disease-free stock. Cross-inoculation tests by DuCharme & Birchfield (1956) have shown that physiological strains of the burrowing nema exist. Longevity studies by Birchfield (1957) have shown that this organism will perish in the soil without food in about 4 months. Host-plant studies by Birchfield & Bistline (1956) have shown that *Crotalaria spectabilis* may be used as a cover crop on lands known to harbor a burrowing nema population.

Investigations are being conducted by Ford (1954a, 1954b) to find a suitable chemotherapeutant to cure valuable citrus plants in place and to find resistant root stocks.

The most extensive effort to contain the burrowing nema and reduce its spread in valuable groves is the "pull and treat" program described by Suit & DuCharme (1953) and Suit *et al.* (1955). This method advocates that all infected trees be removed and all plant parts raked together and burned. The infested area is then treated with a mixture of dichloropropane-dichloropropene (DD) at the rate of 60 gallons or 600 pounds per acre. A waiting period of 2 years is required before the area can be replanted. This treatment is not recommended as a means of eradicating the organism but as the best method known at this time to slow its progress.

Ornamental and citrus nursery stocks infected with the burrowing nema are placed in quarantine by the authority of the Florida Plant Act of 1927 and must be hot-water-treated before being released. No federal quarantine has been imposed but the states of California, Arizona, and Texas have each imposed a quarantine against this nema.

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Control of the Plum Nursery Mite on Sweet Cherries[1]

EDWARD W. ANTHON[2]

During the 1954–56 seasons, experiments were conducted in north central Washington to evaluate new acaricides and to test currently recommended materials for commercial control of the plum nursery mite, *Vasates fockeui* (Nal.). The big-beaked plum mite, *Rhynacus gigantorhynchus* (Nal.), was involved to a lesser degree. Increased damage by these mites since 1954 precipitated a testing program to find effective control measures.

Lime-sulfur or oil-lime-sulfur sprays have always given temporary control of the mites. However, Webster (1949) reported that dormant lime-sulfur has not been sufficient to hold the rust mites in check for the entire season. Experimental spray trials by the author in 1954 verified Webster's results. Where severe infestations of rust mite were observed in growers' orchards, a dormant application of lime-sulfur did not hold them in check. By midseason, mites increased to detrimental proportions. However, a dormant spray of lime-sulfur followed by a summer spray of wettable sulfur have been very effective in controlling this mite on cherries.

With the advent of parathion, dormant sprays were discontinued. Growers who had been conscientiously using parathion regularly had obtained satisfactory control until 1954. However, in some orchards of north cen-

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tral Washington, it has become difficult to obtain satisfactory control of this mite with parathion and other commonly used organic phosphorus compounds. In other orchards these sprays continue to control plum nursery mite, but the residual action was shorter than when the the non-phosphorus compounds were used. It has often been necessary to use non-phosphorus acaricides annually to control heavy infestations of this mite.

These mites are normally surface feeders, preferring the upper leaf surface. However, in severe infestations they may be found on the lower side. Continued feeding causes the leaves to roll longitudinally upward and turn brown. These symptoms may be present over the entire tree. Several hundred mites may occur on a single leaf and, because of their small size, seriously damage an orchard before the grower becomes cognizant of them.

1954 STUDIES.—In 1954 several preliminary pre-harvest control spray tests were conducted against this pest. The first trials consisted of combination sprays of parathion with Aramite or Sulphenone. The results are given in table 1. Both combinations gave good control. In addition, a pre-harvest airplane dust application of 1% TEPP plus 4% Aramite, a total 50 pounds per acre, was used on several orchards with good success.

A post-harvest spray experiment was conducted in a cherry orchard in which the grower had difficulty in controlling this pest with organic phosphorus insecticides. All sprays except demeton were non-phosphates, applied in combination with parathion. Demeton was used alone. Parathion was chosen primarily because of its use for controlling cherry fruit fly. All sprays were applied with orchard guns from conventional ground equipment. Each treatment was applied on July 14 to three randomly located trees. One pre-treatment and four post-treatment mite counts were made on 50 leaves from each tree, collected at random from both treated and untreated trees. These leaf samples were then run through a mite-brushing machine onto a 5-inch circular glass plate. One-tenth of the mites on the plate surface were counted as described by Klostermeyer *et al.* (1956). Results (table 1) show that the combination spray of Aramite 30% at $\frac{3}{4}$ pound per 100 gallons plus parathion 25% at 1 pound per 100 gallons gave good control for 3 weeks, but failed after a month. The combination of Chlorobenzilate 25% at 1 pound plus parathion 25% at 1 pound per 100 gallons gave excellent control for 3 weeks, but mites began to increase in the fourth week. Demeton (21.2%) at 1 or $1\frac{1}{2}$ pints (table 1) was slow in controlling this mite, but after 4 weeks both concentrations were superior to other materials tested.

Table 1.—Control of plum nursery mite on sweet cherries with combination sprays of parathion and non-phosphorus acaricides, Wenatchee, Washington. 1954.

Materials and Amount Used per 100 Gallons of Water	Average Number of Mites per Leaf				
	Pre-treatment	Post-Treatment			
	July 14	July 20	July 26	Aug. 5	Aug. 12
Parathion W.P. (25%) 1 lb. plus:					
Sulphenone W.P. (40%) 1 lb.	327	7.0	56	100	321
Sulphenone Em. (40%) 1 pt.	507	.6	23	40	242
Aramite W.P. (15%) 1.5 lbs.	629	0	15	13	57
Aramite W.P. (30%) 0.75 lb.	594	0	6	3	22
Sulphenone W.P. (40%) 2 lbs.	446	.6	13	16	66
Chlorbenside W.P. (20%) 2 lbs.	194	28	110	76	227
Chlorobenzilate W.P. (25%) 1 lb.	328	1	0	0	15
Demeton Em. (21.2%) 1.5 pts.	262	16	20	6	5
Demeton Em. (21.2%) 1 pt.	155	5	4	2	3
Untreated check	17	17	123	24	336

Table 2.—Post-harvest spray applications for the control of plum nursery mites on cherries, Wenatchee, Washington. 1955.

Materials and Amount Used per 100 Gallons of Water	Average Number of Mites per Leaf				
	Pre-treatment	Post-Treatment			
	Aug. 17	Aug. 25	Sept. 1	Sept. 8	Sept. 15
Parathion W.P. (25%) 1 lb.	198	0	0	15	43
Parathion W.P. (25%) 1 lb. plus:					
Aramite W.P. (15%) 1.5 lbs.	94	.2	.4	2	30
Sulphenone W.P. (40%) 2 lbs.	247	.2	1	1	4
Sulphenone Em. (40%) 1 qt.	232	.2	.8	2	15
Am. Cyanamid 12008 Em. (48.5%) 0.375 pt.	364	.2	0	0	5
Chlorobenzilate W.P. (25%) 1 lb.	332	0	.4	2	1
Kelthane W.P. (18.5%) 2 lbs.	463	0	0	.2	0
Kelthane Em. (18.5%) 1 pt.	262	.2	32	11	58
Thimet Em. (47.5%) 1 pt.	284	0	0	.8	10
Trithion Em. (37%) 1 pt.	132	0	0	2	14
Trithion W.P. (25%) 1 lb.	421	0	0	1	14
Hercules AC-528 W.P. (25%) 1 lb.	159	0	0	1	7
Hercules AC-528 Em. (50%) 1 pt.	99	0	0	0	3
Sulfur W.P. (85%) 3 lbs.	518	0	0	0	6
Sulfur W.P. (85%) 4 lbs.	362	.2	0	.2	0
Phosdrin Em. (25%) 1 pt.	288	0	0	4	40
Guthion W.P. (25%) 0.5 lb.	131	0	.4	9	32
Demeton Em. (21.2%) 1.5 pts.	241	0	0	1	.4
Malathion 1 pt.	319	1	3	9	54
Untreated check	360	62	12	11	8

An airplane application of 3 pints of demeton per acre was applied August 28, 1954 to an 8-acre orchard of old cherry trees when there was very little new growth. Leaf sampling and mite counts made as previously described showed only 9% reduction of the population.

1955 STUDIES.—A new orchard site was selected for the 1955 tests which showed that parathion gave good control of this mite for 2 weeks (table 2). A number of acaricides were tested with parathion added to control the cherry fruit fly. Four randomized single-tree replicates were used for each treatment. Sprays were applied August 18 to 23 with the same equipment and in the same manner as in 1954. The evaluation of the treatments was made by the same technique employed in 1954.

The untreated trees in table 2 show a reduction from 360 mites per leaf on August 17 to 8 mites per leaf on September 15. This condition has been found before in previous spray experiments, but no reliable information has been found to explain why this sometimes happens with Eriophyid mites. The 1955 post-harvest spray trials (table 2) showed that the following sprays—Kelthane plus parathion, sulfur plus parathion, Hercules AC-528 plus parathion, or demeton used alone—gave excellent control for 1 month.

1956 STUDIES.—On May 26 an additional experiment was conducted to determine the effect of some of the promising non-phosphorus insecticides at various concentrations. Two new organic phosphorus compounds, Hercules AC-528 and Trithion, were included to re-evaluate their performance. These acaricides were applied to large cherry trees using conventional ground equipment. Four randomized single-tree replicates were used for each treatment. One pre-treatment and four post-treatment mite counts were made. Twenty-five leaves from each

Table 3.—Spring spray application for control of plum nursery mite on cherries, Malaga, Washington. 1956.

Materials and Amount Used per 100 Gallons of Water		Average Number of Mites per Leaf				
		Pre-Treatment	Post-Treatment (June)			
		May 23, 24	1	8	15	22
Aramite	2 lbs.	15	0.0	0.8	1	2
Aramite	1 lb.	17	.6	1	2	4
Sulphenone	1 qt.	10	0	1	3	7
Wettable Sulfur	2.5 lbs.	13	2	2	4	5
Wettable Sulfur	3 lbs.	18	.2	1	2	3
Kelthane	1 lb.	12	.2	1	1	5
Kelthane	1.5 lbs.	13	.7	.5	.7	1
Kelthane	2 lbs.	13	.2	1	2	4
Parathion	1 lb.	12	.3	8	9	19
Chlorobenzilate	1 lb.	9	.9	.5	2	2
Chlorobenzilate	1.5 lbs.	11	.4	0	.7	1
Hercules AC-528	1 lb.	6	2	.6	.3	1
Trithion	1 pt.	13	.1	.7	1	1
Untreated Check		9	.1	11	20	24

tree were collected at random from treated and untreated trees and counted as previously described.

This experiment showed that Kelthane, Chlorobenzilate, Trithion, Aramite, wettable sulfur, and Hercules AC-528 all gave good control for 1 month. The detailed results are given in table 3.

MISCELLANEOUS OBSERVATIONS.—Because the new non-phosphorus acaricides were not registered for use on cherries, a number were sprayed on cherries from May 11 to May 28 to determine the amount of visible residue on cherries when harvested the last week of June. The following materials were used: wettable sulfur, Sulphenone, Aramite, Kelthane, and Chlorobenzilate. Observations were made 3 weeks after application and again at harvest. Cherries sprayed with wettable sulfur, Aramite, Kelthane, and Chlorobenzilate showed little visible residue 3 weeks after application and less at harvest in the last week of June.

In the past, good results have been obtained using aerial dust applications of 4% Aramite and 1% TEPP. In 1956 additional airplane applications of dusting sulfur 98% at 50 pounds per acre and TEPP alone were made to cherry orchards. Pre-treatment and post-treatment mite counts were then made to determine their efficiency. Aerial applications of sulfur gave good results, but were not as good as conventional ground applications. TEPP aerial dust applications gave erratic control.

SUMMARY.—In extensive experimental trials during 1954–56 a number of acaricides gave good control of the plum nursery mite, *Vasates fockeui* (Nal.). One of the best materials was wettable sulfur. Delayed dormant sprays of lime-sulfur were also effective, but did not give seasonal control. However, all of the following materials have given good control for 1 month: Chlorobenzilate, demeton, Kelthane, Aramite, Sulphenone, wettable sulfur, Trithion, Thimet, and Hercules AC-528. Chlorobenzilate, Kelthane, Aramite, and Sulphenone, if applied 5 weeks before harvest, showed no excessive visible residues.

Aerial dust applications of 4% Aramite plus 1% TEPP also gave good control. TEPP dust alone gave erratic results.

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Biology and Control of the Citrus Flat Mite[1]

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The citrus flat mite was first observed by H. C. Lewis in 1944, on lemon trees damaging lemons near Porterville, Calif. Lewis sent specimens to E. A. McGregor, who published the first description in 1949 and named the mite *Brevipalpus lewisi*. Ebeling & Pence (1949) reported damage by this mite to pomegranates in the Porterville area. It has since been found on oranges, grapefruit, and tangerines. This mite is now also a potential pest of walnuts in California (Michelbacher 1956) and has been found on grapes near Yuma, Arizona.

Citrus flat mite infestations were found only occasionally in citrus groves in central California from the time it was first recognized in 1944 until 1952. High populations causing some injury to the fruit were found in a few groves during the summer of 1952. Since then there has been a general increase in the economic importance of this mite throughout the citrus-growing area of central California. Injury has been less extensive but equally severe in numerous groves in the Coachella and Moreno valleys of southern California, and some very heavy infestations were found scarring citrus in the Imperial Valley in 1955.

The reasons for the sudden increase in populations of this mite are not completely known, but the rise in its importance on citrus in central California is closely correlated with the reduction in use of sulfur, lime-sulfur, and oil spray applications. Sulfur and oil have both been found to be toxic to this species, whereas the chlorinated hydrocarbon or phosphate insecticides used to replace oil and sulfur for the control of pests on citrus do not effectively reduce injurious populations. The mite population increases in the Imperial, Coachella, and Moreno valleys are believed to be more closely related to ecological changes rather than insecticidal use, since these areas have not been under continuous insecticidal control programs.

Other species of the genus *Brevipalpus* also found on citrus in California are principally *B. californicus* (Banks),

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B. australis (Tucker), and *B. inornatus* (Banks); but there have been no indications of serious injury to citrus trees or fruit by these three species.

It is the purpose of this report to present information obtained on the life history of the citrus flat mite and to summarize results obtained with experimental treatments of various insecticides under field conditions in California.

LIFE HISTORY.—Citrus flat mites are very small, flat, and slow moving, and are found most abundantly at the stem end of the fruit near or under the fruit button. They may have a small dark area dorsally but their overall color varies from a light reddish brown to a rather bright red. The bright red mites are particularly evident in the young immature forms. Eggs are laid singly in cracks and crevices of the fruit, twigs, and leaves and are pink to red in color and oval in shape.

Observations indicate that the mites prefer green fruit to ripe fruit. Fruit in any stage of growth is preferred to leaves and twigs. The population is greatly reduced at harvest time since most of the mites are on the fruit. Consequently, trees bearing fruit that is held on the trees for late picking support injurious populations for more of the year than trees bearing fruit that is picked early.

In central California this mite species probably overwinters only in the adult stage as no eggs were observed on fruit, leaves, or twigs from October to mid-April. In the Imperial and Coachella Valley citrus-growing areas of southern California, eggs, adults, and immature stages were found in every month of the year. The number of generations developing in the course of a year has not been determined. Peak populations are reached during the warmest months. Periods of extremely high temperatures and low humidity are not effective in reducing populations of this mite as of citrus red mite, *Metatetranychus citri* (McG.), and Yuma mite, *Eotetranychus yumensis* (McG.).

FRUIT INJURY.—The injury caused by this mite on lemons (Lewis 1944) is, in general, typical of that observed on all citrus varieties. It has also been observed that the discolored areas on young fruit, which are usually attributed either to leafhopper feeding or to oviposition by citrus thrips, are areas where the citrus flat mites congregate. On fruit that is free of mites these spots tend to disappear as the fruit color changes from green to orange; however, in groves having even a light population of this mite some of the mature fruits show a depression in these small isolated circles and a scablike scar (fig. 1). These scars are secondary injuries caused by this mite. Fruit scars caused by other insect pests, by rubbing of branches and twigs, or by mechanical equipment, etc., also are areas where mites congregate. Damage consists almost entirely of a reduction in grade of fruit (fig. 2). No injury has been observed on the leaves or wood of trees infested with citrus flat mite.

INSECTICIDE STUDIES.—This mite was found to be most economically controlled by sulfur applied as preventive treatments during late winter and early summer. Tests in 1952 using dusting sulfur at the rate of at least 75 pounds per acre, or thorough-coverage sprays of wettable sulfur

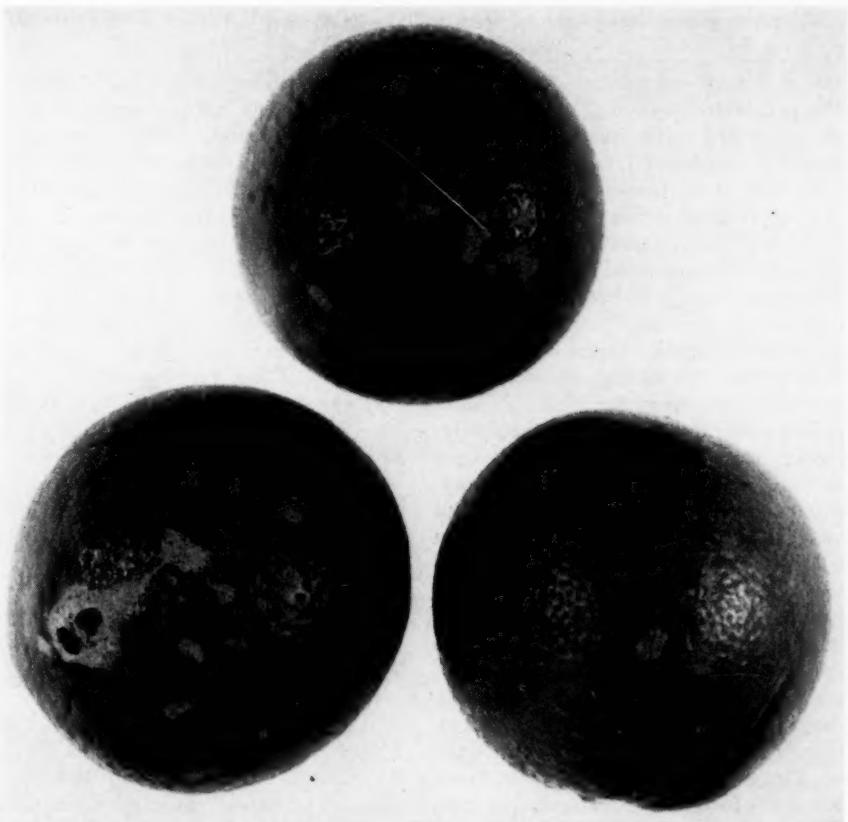

FIG. 1.—Injury to navel oranges caused by citrus flat mite showing scablike scars formed in isolated depressions.

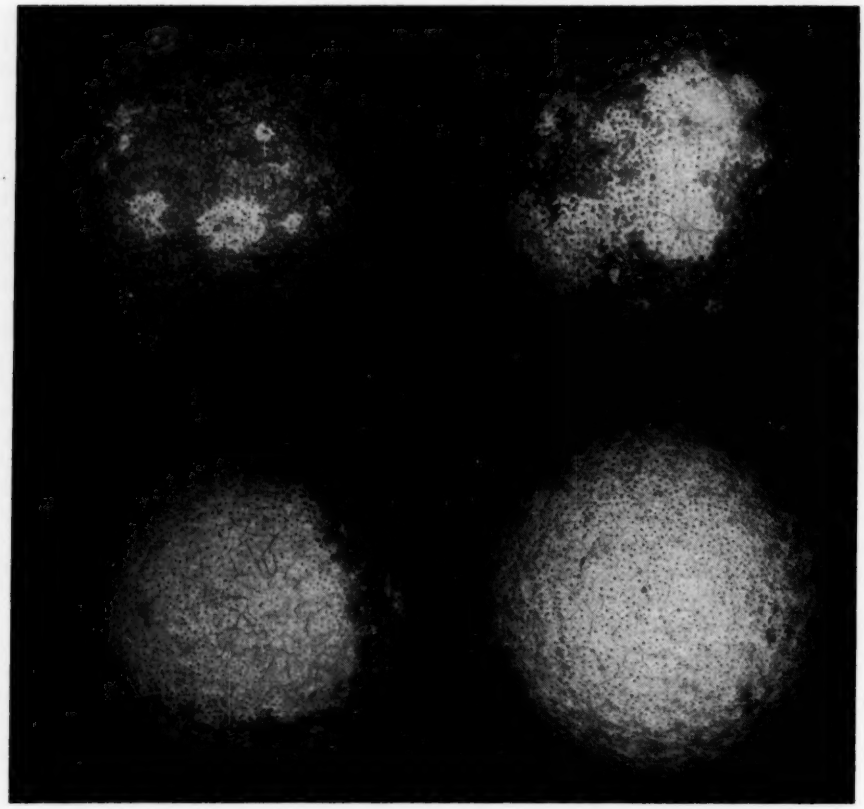

FIG. 2.—Injury to Valencia Oranges caused by citrus flat mite showing the degree of injury found on fruit.

applied at the rate of 4 to 6 pounds per 100 gallons, generally resulted in satisfactory seasonal control. However, sulfur cannot be applied safely during or immediately preceding periods of hot weather, or within 60 days of an oil application. Therefore, treatments other than sulfur are needed for the control of this mite.

MATERIALS AND METHODS.—Experiments were conducted in 1955 and 1956 in commercial citrus groves to evaluate the effectiveness of a number of acaricides. Three types of applications were used in these studies: (1) thorough-coverage sprays applied at the rate of 15 to 25 gallons per tree with conventional high-pressure spray equipment using manually operated hand guns; (2) high-volume sprays applied with high-pressure mechanically-driven oscillating booms at the rate of 1000 gallons per acre; (3) variable volume sprays applied with spray-blower equipment at the rate of from 250 to 1000 gallons per acre. The spray-blower is a multi-nozzled, air-blast type of mechanical equipment modified from the original spray-duster (Lewis & Landon 1949).

Plots were replicated within groves when possible. The unit used for evaluating the mite population density was 1 square inch of surface near the stem end of the fruit, where the mites are generally found in greatest numbers. The average number of live mites per square-inch unit on eight randomized fruits per tree was determined with the aid of a hand lens. Plot size ranged from 2 to 16 trees, and results reported in the tables represent averages from replicated plots. The number of mites necessary to cause injury to the fruit and consequently warrant treatment has not been determined, but observations on the injury caused by this mite indicate that, according to this method of evaluation, the occurrence of more than 0.1 mite per unit prior to the time fruit color changes from green to orange will probably cause some degree of injury to the fruit.

RESULTS.—A number of insecticides were initially evaluated on navel orange trees in three groves in Tulare County. The results of these tests are shown in table 1. It became apparent from these results, and also from observations on commercially sprayed groves having populations of citrus flat mite, that the organic phosphate compounds did not reduce mite populations to the low level desired. The two chlorinated hydrocarbon compounds Kelthane and Chlorobenzilate gave the best results. Dosages of 18.5% Kelthane wettable powder ranging from 0.4 pounds to 1.2 pounds per 100 gallons gave effective reduction of mites in all three groves throughout the fruit-growing season. A formulation of 25% Chlorobenzilate wettable powder was equally effective in grove B; but at a dosage of 1.0 pound per 100 gallons in grove A and 1.2 pounds in grove C the mite population was increasing by October of the same year, 128 and 89 days, respectively, after treatment.

Experiments with various acaricides on tangerines in Imperial County were begun in November, 1955 (table 2). Results with Kelthane and Chlorobenzilate were similar to those obtained with these compounds on oranges in Tulare County. Kelthane-treated plots were exceptional, however, because of the low mite populations

Table 1.—Relative effectiveness of several wettable powder acaricides applied for control of the citrus flat mite on navel orange trees in Tulare County, Calif., 1955.

Formulation	Pounds per 100 Gallons Water	Average No. of Live Mites per Unit of Fruit Surface[a] March 3	Oct. 12
Grove A (Treated June 16)[b]			
Phostex, 25%	1	1.5	0.2
	2	1.1	.3
Trithion, 25%	1	0.4	.3
	2	.9	.1
Hercules AC-528, 25%	1	.4	.1
	2	.4	.2
Diazinon, 25%	1	.3	.1
	2	.6	.3
Kelthane, 18.5%	1	.0	.0
	2	.0	.0
Chlorobenziliate, 25%	1	.0	.1
Grove B (Treated July 8)[c]			
Trithion, 25%	1	2.5	—[d]
Hercules AC-528, 25%	1	2.0	—[d]
Diazinon, 25%	1	3.6	—[d]
Kelthane, 18.5%	1	0.0	0.0
Chlorobenzilate 25%	1	0.0	0.0
Grove C (Treated July 15)[c]			
Chlorobenzilate, 25%	0.6	0.0	0.5
	1.0	.0	.6
	1.0	.0	.6
	1.2	.0	.1
Kelthane, 18.5%	0.4	.0	.01
	0.6	.0	.0
	1.2	.0	.0

[a] Unit, 1 square inch of fruit surface near the button.
[b] Applied with conventional sprayer at the rate of 2250 gallons per acre (thorough coverage).
[c] Applied with mechanical oscillating boom at the rate of 1000 gallons per acre.
[d] Re-treated.

found in June of the following year. Plots sprayed with Genite had no mites 1 month after treatment, and those sprayed with Chlorbenside were free of mites until the following June, 8 months after treatment.

Dosage and spray-volume tests were made during the navel orange growing season in four central California groves. Results of treatments applied at the time of the year when sulfur applications are possible are shown in grove D, table 3. Kelthane and Chlorobenzilate gave longer seasonal control of citrus flat mite than the recommended dosage of 75 pounds of sulfur per acre. Different volumes of spray per acre were evaluated in grove E, using 5 and 10 pints of each of these two materials (18.5% Kelthane and 25% Chlorobenzilate both as emulsifiable concentrates). Best results with Chlorobenzilate were obtained with dosages of 10 pints in at least 500 gallons of water per acre. Kelthane was equally effective at 10 pints in 250 gallons of water per acre. Kelthane at 5 pints in 500 gallons, when applied to plots in grove F, was as effective in reducing populations of these mites as Kelthane at 10 pints in 250 gallons.

Table 2.—Relative effectiveness of several acaricides applied for control of the citrus flat mite on tangerine trees in Imperial County, Calif. 1955–56.

Formulation[a]	Amount Toxicant per 100 Water	Average No. of Live Mites per Unit of Fruit Surface[b] Pre-Treatment	Nov. 23, 1955	Dec. 7, 1955	June 26, 1956
Kelthane, 18.5% W	2 oz.	39.8	1.0	0.0	0.6
	4 oz.	38.6	0.2	0.0	0.1
	8 oz.	41.0	0.1	0.0	0.0
	12 oz.	51.3	0.0	0.0	0.0
Chlorobenzilate, 25% W	8 oz.	32.3	1.3	0.1	10.6
	16 oz.	21.8	0.5	0.0	8.5
Genite, 25% EC[c]	1 pt.	26.8	0.5	0.0	11.3
	1 qt.	19.5	0.2	0.0	1.5
Trithion, 25% W	16 oz.	10.3	2.0	0.4	15.4
	32 oz.	22.0	0.9	0.3	10.1
Chlorbenside, 20% EC	2.5 pt.	15.5	0.03	0.2	0.0
Ovex, 50% W	16 oz.	—	2.3	0.4	2.3

[a] Applications made Nov. 2, 1955, with conventional sprayer at the rate of 2000 gallons per acre. W = wettable powder; EC = emulsifiable concentrate.
[b] Unit, 1 square inch of fruit surface near the button.
[c] *p*-Chlorophenyl benzene sulfonate; General Chemical Co., New York, N. Y.

Applications designed to ascertain the lowest dosage of Kelthane needed to keep populations sufficiently low to prevent fruit injury were evaluated in grove G. Plots sprayed with 4 pints Kelthane in 500 gallons of water had resulted in no measureable mite population for 3

Table 3.—Effect of dosages of Kelthane and Chlorobenzilate and spray volumes on control of the citrus flat mite on navel orange trees in four groves in Tulare County, Calif. 1956.[a]

Formulation[b]	Amount Toxicant per Acre	Gallons per Acre	Average No. of Live Mites per Unit of Fruit Surface[c] Pre-Treatment	Aug. 14	Sept. 28	Nov. 6
Grove D (Treated May 18)						
Kelthane	6.75 pts.	250	—	0	0	0
	10 pts.	250	—	0	0	0
Chlorobenzilate	10 pts.	250	—	0	0	0
Sulfur (wettable)	75 lbs.	500	—	0	0.5	0.9
Grove E (Treated August 13)						
Kelthane	5 pts.	500	1.9		1.0	0.1
	10 pts.	500	3.1		0	0
	10 pts.	100	2.8		0	0
	10 pts.	250	1.7		0	0
Chlorobenzilate	5 pts.	500	1.3		0.03	0.3
	10 pts.	500	1.9		0.01	0
	10 pts.	100	1.5		0.01	0
	10 pts.	250	1.3		0.02	0.02
Grove F (Treated August 20)						
Kelthane	5 pts.	250	—		0.02	0
Chlorobenzilate	5 pts.	250	—		0.1	1.9
Kelthane	10 pts.	250	—		0	0
Chlorobenzilate	10 pts.	250	—		0.01	0
Kelthane	5 pts.	500	—		0	0
Grove G (Treated August 27)						
Kelthane	1 pt.	500	1.8		0.3	0.7
	2 pts.	500	1.7		0.1	0.02
	4 pts.	500	1.6		0	0
	8 pts.	500	1.6		0	0
	10 pts.	500	1.7		0	0
Chlorobenzilate	5 pts.	500	1.4		0.3	1.2

[a] Applications made as a mist spray with spray-blower.
[b] Kelthane, 18.5% emulsifiable concentrate; Chlorobenzilate, 25% emulsifiable concentrate.
[c] Unit, 1 square inch of fruit surface near the button.

months after application, and dosages as low as 2 pints of this Kelthane formulation were more effective than 5 pints of Chlorobenzilate at the same amount of spray volume.

SUMMARY.—Observations of the life history of the citrus flat mite, *Brevipalpus lewisi* (McG.), on citrus in California were made over a 2-year period. In central California this mite overwinters as an adult; in southern California all stages of the mite were found during the winter. This species is not adversely affected by hot, dry periods of the year. The primary injury consists of corky, scab-like spots which lower the grade of the fruit. Evaluation of the mite populations was determined by using 1 square inch of fruit surface at the stem end and examining with a hand lens. Eight fruits per tree and from 2 to 16 trees per plot were used as a randomized sample.

Chemical control experiments indicated that organic phosphorous insecticides as a class are ineffective in reducing the numbers of this mite to a point where injury will not occur. Kelthane and Chlorobenzilate were the most effective insecticides tested. A dosage of 5 pints of 18.5% Kelthane emulsifiable concentrate in 250 gallons of water per acre was as effective as a dosage of 10 pints of 25% Chlorobenzilate emulsifiable concentrate in 250 gallons per acre in reducing populations of this mite.

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The Effects of Certain Fungicides on Honey Bees[1]

E. J. ANDERSON, F. R. SHAW and D. J. SUTHERLAND

In recent years in many of the northeastern states, recommendations for the control of apple scab have included an application of a fungicide during bloom. Such recommendations have caused concern to beekeepers and in a few instances to those who proposed the inclusion of such materials. Since no practical work had been undertaken in recent years to determine the effects of such treatments, the authors have conducted a series of tests to attempt to evaluate the possible hazards of such a recommendation.

Shaw (1941) summarized the information available up to that time. In 1942, Shaw & Bourne reported that the application of sulfur during bloom could result in the development of dysentery in bees and that there was some reduction in the length of life of bees exposed to this fungicide. They also indicated that the application of a sulfur spray or dust seemed to repel bees for a period of about 24 hours.

Shaw (1952) conducted tests under field conditions to determine the hazards of the application of phenylmercury lactate (puratized) to bees. In these experiments bees were exposed to the contact action and also to the residual action of the fungicide applied at the rate of 1 pint per 100 gallons of water. At this rate, there was no significant difference in the mortality of the treated bees and the checks at the end of a 10-day period. In a second test, bees were exposed to varying concentrations (1X to 4X) of the fungicide applied as a contact. Not until the 4X concentration was reached was there any significant difference in the mortality rate of the bees. At the end of 10 days, 12.2% of the bees exposed to the 4X concentrations had died, whereas the mortality from the other applications and the check did not exceed 1%. It was concluded that the use of phenylmercury during bloom had little effect on bees, at least under the conditions tested.

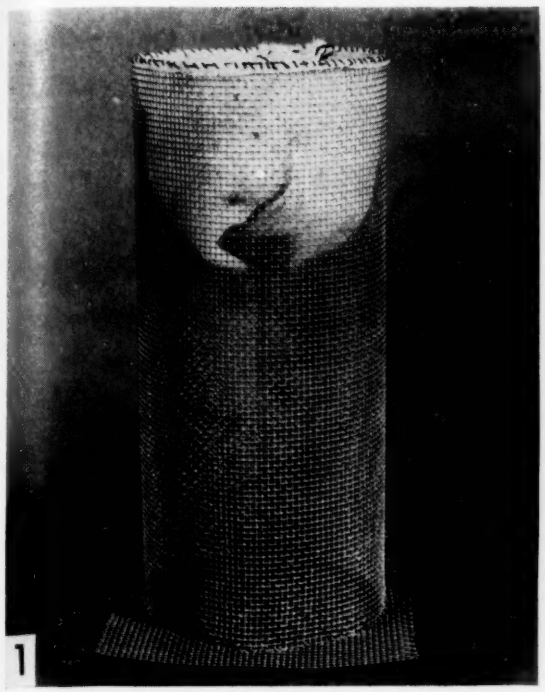
PRELIMINARY TESTS.—In 1955, Shaw and Coxeter conducted some preliminary tests of the effects of fungicides on honeybees. The procedure followed was to remove worker bees from brood frames by shaking them into a pail. The bees were then placed in sleeve cages (fig. 1) and taken to the orchard. To determine the effects of the fungicides as contact agents, cages of bees were fastened to twigs bearing leaves and blossoms and then were sprayed. To determine the effects of the residues of the treatment, twigs were sprayed, allowed to dry and then caged bees were exposed to the residues for 20 minutes. Following these treatments the bees were taken to the laboratory, transferred to clean cages and provided with sugar syrup. The cages were placed in a darkened room at a temperature of 60 to 70° F.

In these preliminary tests, all applications were made by hand using a compressed-air sprayer. The cages were sprayed until the foliage and the bees appeared to be wetted, but we did not spray sufficiently long to permit run-off. The fungicides included in this series of tests included: Captan at the rate of 2 lbs. per 100 gallons, ferbam at the rate of 1½ lbs. per 100 gallons, glyodin at the rate of 1 quart per 100 gallons, and Puratized at the rate of 1 pint per 100 gallons. The caged bees were examined daily, and the number of dead recorded. The data are given in table 1. Results of these preliminary tests indicated that, with the exception of glyodin as a contact spray, the fungicides used in these tests were not a serious threat to bees.

In 1955, Sutherland[2] conducted preliminary laboratory tests of the contact action of pesticides using honeybees collected in the field. Glyodin at 1 quart per 100 gallons

[1] Accepted for publication February 12, 1957.

[2] Research carried on under a grant from the Massachusetts Society for Promoting Agriculture.



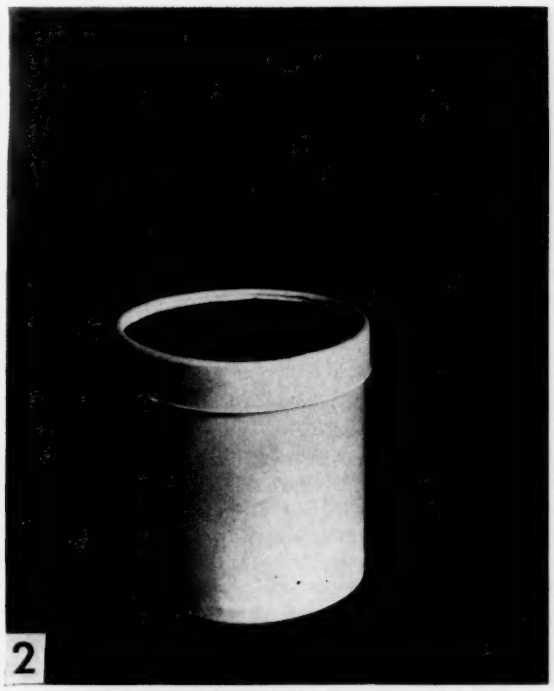


FIG. 1.—Sleeve cage used to confine honeybees to twigs bearing leaves and blossoms in the orchard.
FIG. 2.—Improved cage for use in studying the effects of pesticides on honeybees.
FIG. 3.—Honeybees being placed into 5-mesh wire cages for spraying with power sprayer.
FIG. 4.—Clean cage to which sprayed honeybees were transferred. It contained sugar syrup, water, and a 2-inch-square piece of honeycomb.

Table 1.—Days required to produce 50% mortality of honeybees by certain fungicides.

Captan	
Contact	21
Residue	23
Ferbam	
Contact	21
Residue	23–24
Glyodin	
Contact	1– 2
Residue	20–21
Puratized (phenylmercury lactate)	
Contact	22
Residue	19–20
Untreated	21

was sprayed through the caged bees for 10 seconds at the rate of 11 ml. per 10 seconds, an amount observed to wet all bees in the cage. After treatment the bees were transferred to clean cages and placed in darkness at 60° F. Results of these tests indicated that under such conditions and dosage, glyodin might be toxic to field honeybees. Sutherland also developed an improved cage (fig. 2) for the studies of the effects of pesticides on bees. He used a quart ice cream carton 4″ in diameter having the ends replaced with 8-mesh screen. These are easily made and are disposable.

In order to learn whether other workers had any experience with the effects of glyodin on honeybees, we brought our preliminary data to the attention of a group of fruit spray specialists. As a consequence, representatives of the manufacturers of glyodin requested the senior author to conduct a series of tests using power equipment under field conditions.

The bees sprayed by Anderson were shaken into two sets of 5-mesh wire cages (fig. 3) and sprayed with a Bean power take-off spray gun operated at 500 p.s.i. The bees were sprayed with the nozzle of the spray gun held at a distance of 8 feet from the cages. The spray was passed four times across the bees at which time they appeared to be covered with the spray material. The bees were then transferred to 10 clean cages (fig. 4) each containing sugar syrup, water and a 2-inch-square piece of honey comb on which they clustered. One set of cages was kept at room temperature about 72 to 78° F. The second set was kept in the dark in a constant temperature chamber held at 88 to 90° F. The dead bees were removed daily through a trap door in the bottom of the cages and recorded. The fungicides used and the results obtained are given in table 2. The mortality rate in cages kept in the light of the open room and at the lower temperatures of approximately 76° F., was more than twice as high as it was in the dark constant temperature chamber held at approximately 89° F. There was no significant difference in the mortality rate between the check cages and any of those treated with fungicides.

The bees in a third set of 10 cages of the type provided with two bottles and honey comb were sprayed in the cages. The spray materials wetted the screen and parts of the cages, bottles, and comb so that the bees came in contact with the dried spray material. The sprayed bees were

Table 2.—Per cent mortality, over a 15-day period, of honeybees sprayed with certain fungicides.

Material	Rate per 100 Gallons	Total Mortality of Bees	Bees Still Alive
At room temperature, 72 to 78°F.			
Glyodin	1 qt.	16	194
Glyodin +phenylmercury	1 pt. 0.5 pt.	18	149
Captan	2 lbs.	22	100
Check		30	106
In a dark chamber at a temperature of 88 to 90° F.			
Glyodin	1 qt.	7	131
Glyodin +phenylmercury	1 pt. 0.5 pt.	9	158
Captan	2 lbs.	5	140
Check		7	115

left in these cages for the 15-day period. Results were similar to those shown in table 2.

It is evident that there is a lack of agreement between the data presented by Shaw and Coxeter and that obtained by Anderson. Since the only apparent differences in the two series of tests were the methods of application, additional experiments were conducted during 1956.

TESTS IN 1956.—In the first test, caged bees were exposed to an application of glyodin at the rate of 2 quarts per 100 gallons applied by a commercial Cardox speed sprayer. The treated bees were removed to clean cages, fed and held under conditions similar to those of our preliminary tests. On the basis of the number of days to produce 50% mortality, there was practically no difference between the treated and untreated bees.

In the second test, applications of glyodin at the rate of 1 quart per 100 gallons were made using a Bean hydraulic orchard sprayer with a Hardie boom, and with a Cardox speed sprayer. In addition, an application of water alone was made with the hydraulic sprayer to determine if any toxicity resulted. The data appear in table 3. From these data, we concluded that under the conditions of the experiment, glyodin, at the rate of 1 quart per 100 gallons of water, produced little, if any, serious effects on bees.

In 1956, Sutherland also carried out further experiments. In order to attempt to secure bees of the same ages as would normally be found in the field, he selected incoming field bees having loads of pollen. Such bees were

Table 3.—Effects of glyodin at 1 quart per 100 gallons on honeybees.

Type of Treatment and Cage Location	Days to Produce 50% Mortality
Speed sprayer	
Periphery of tree	17–18
Near center of tree	17–18
Hydraulic sprayer	
Periphery of tree	15–16
Near center of tree	17–18
Water alone	18
Checks	19–20

Table 4.—Number of days required to produce 50% mortality of honeybees by certain fungicides.

Material	Rate per 100 Gallons	Treated	Control
By contact action			
Captan	2 lbs.	11	9
Glyodin	1 qt.	9–11	9
Fermate	1.5 lbs.	11–12	14
Puratized (phenylmercury acetate)	1 pt.	7–8	7
Sulfur	6 lbs.	7–8	9
As stomach poisons			
Captan		7–9	10
Glyodin		7–11	12
Fermate		11–12	15
Puratized (phenylmercury lactate)		5–8	5–7
Sulfur		7–10	12

placed in cages, sprayed in the laboratory, transferred to clean cages and held in a dark room at 65° F. These data relative to the contact action of some of the more common fungicides are given in table 4. Sutherland also conducted some tests to attempt to determine the effects of the same fungicides as stomach poisons. Pollen-gathering bees were selected, placed in cages and fed a mixture of 1 part sugar syrup to 1 part fungicide solution by weight. These results also appear in table 4. The apparent variation in the length of life of both check and treated bees in some tests can be explained by the fact that not all of the treatments were made at the same time.

In further investigation, Sutherland conducted some laboratory tests to determine the effects of various dosages and concentrations of glyodin on bees. Concentrations of 1 to 10 times the normal amounts (1 quart per 100 gallons) were used in one series of tests. Dosages of 1 to 4 times the amount normally used in the laboratory were included in another group.

An increase in concentration up to 5X did not materially increase the mortality. When dosages of 3 to 4 times normal were applied, mortality increased. It may be that the amount of spray applied, possibly as a measure of the length of exposure, will be found to be the explanation of the mortality encountered during our tests in 1955.

TESTS IN MICHIGAN.—Subsequent to the completion of the investigations in 1956, the authors had an opportunity to examine the data obtained by Parsons & Martin (1956) who reported on their investigations on the effects of 14 fungicides on honeybees, tested as stomach poisons and as contact poisons. These workers concluded that, on the basis of laboratory feeding tests, two phenylmercury compounds, Puratized and Tag 331, killed 50% of the bees within 24 hours. Glyodin caused a toxicity of 50% under similar conditions in 48 hours. In laboratory contact tests, Puratized killed 50% of the bees within 48 hours. Tag 331, glyodin, zerlate and Coro SDD killed 50% within 4 days. Pomasol, Dithane Z-78, Crag 658, Coromerc, Phix, manzate, fermate, phygon and Orthocide had no contact action. Under field test conditions, both Puratized and Tag 331 were poisionous to bees. Glyodin appeared to be less toxic than in the laboratory tests. Orthocide, Phix and Coromerc showed moderate toxicity. Phygon and manzate appeared to be toxic in field cage tests.

CONCLUSIONS. On the basis of tests conducted in Massachusetts and in Pennsylvania, none of the following fungicides caused any serious mortality to honeybees under the conditions tested: glyodin, fermate, Puratized, captan and sulfur. Such tests included effects of these materials as contact poisons and as residuals under field conditions. Some preliminary tests were made using the materials as stomach poisons.

From data obtained by workers in Michigan, phenylmercury acetate compounds appear to be quite toxic to bees, both as contact and stomach poisons. Glyodin appeared to be somewhat toxic under laboratory conditions as a stomach poison and to a lesser degree as a contact poison. Under field conditions, its importance as a contact poison appeared to be less significant than it was in the laboratory.

Further investigations are necessary before attempting to evaluate the effects of many of the newer fungicides on honeybees. Such investigations should include the effects of the chemicals as stomach or contact poisons, as residuals, and also of their possible repellent action. Investigations of improved methods of testing the effects of pesticides on honeybees under field conditions are needed.

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Authors are urged to consult the Publication Policies and Manuscript Rules and Suggestions (Vol. 9(4): 575–7) before preparing manuscripts intended for publication in the JOURNAL OF ECONOMIC ENTOMOLOGY.

Granulated Insecticides for the Control of the Alfalfa Weevil[1]

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The damage caused by the alfalfa weevil, *Hypera postica* (Gyll.), in reducing hay yields in western South Dakota has made the application of chemical controls a regular spring practice in the more heavily infested areas. While certain insecticides in the spray form have provided good control for the weevil, as reported by Hastings & Pepper (1951), the field type sprayer has several objectionable features when used in the early spring. The successful use of granulated insecticides for the control of other insects prompted the trial of this type of insecticide application for the alfalfa weevil as a means of overcoming some of the objections to spraying. Lilly (1954) showed the effectiveness of granulated insecticides for corn rootworm control. *Tabanus* larvae were controlled by granulated insecticides as reported by Hansens (1956). Cox *et al.* (1956) found granulated insecticides effective in European corn borer control. Weaver (1956) reported effective control of the meadow spittlebug using various granulated insecticides. Seed weevils on crimson clover were controlled with granulated insecticides as reported by Beckham (1956).

Methods and Materials.—An experiment was initiated in 1956 to compare the effectiveness of granulated insecticides applied in late winter and recommended spray applications in early spring for the control of the alfalfa weevil. Two alfalfa fields were selected, one in Lawrence County and the other in Butte County in South Dakota. Each field was laid out in randomized complete blocks with three replications. Individual plots were 1 acre in size.

The granulated insecticides tested were heptachlor and dieldrin on attaclay. Granulated insecticides were applied at rates of $\frac{1}{4}$ and $\frac{1}{2}$ pound of actual insecticide per acre. The spray applications were of the same insecticides in the emulsion form at the rate of $\frac{1}{4}$ pound of actual insecticide per acre.

In Butte County 21 acres of an alfalfa field which had not been treated in 1955 were selected on the William Schuft farm. The granulated insecticides were applied with an Ezee Flow-100 spreader on March 16. The field at the time of application was covered with snow varying from 1 to 2 inches in depth. The temperature was 27° F. The emulsifiable concentrates were applied on April 17 when the plant growth averaged about 1 inch in height. A field type ground sprayer was used for the spray applications.

Due to the late spring, larval damage was just beginning to appear on June 11 at the $\frac{1}{4}$-bloom stage. Since most of the larvae were in the second and third instars, it was estimated that a severe reduction in hay quality and a distinct reduction in yield would have resulted if mowing had been delayed 10 days. Ten sweeps in each plot with a 15-inch net were made to determine larval populations. Five randomly selected hay samples of yard-square areas were cut in each plot. The hay samples were weighed at the time of cutting and again when air dried. The dry samples were weighed on July 23. The field hay yields were determined from these samples.

In Lawrence County, 21 acres of an alfalfa field which had been treated with $\frac{1}{4}$ pound of actual dieldrin per acre in 1955 were selected. This field, seeded to Cossack alfalfa in 1950, was located on the John Ward farm. The granulated forms were applied on April 13 with a Brillion packer-seeder. The temperature was 47° F. The emulsifiable concentrates were applied on April 14 in 10 gallons of water per acre with a broad-jet sprayer mounted in a pick-up truck. The plant growth on the field averaged 1 inch in height.

Hay and larval samplings were taken on the same days and in the same manner as those in Butte County. Field conditions were also similar as far as larval damage and stage of growth were concerned.

Results.—The data presented in table 1 compare the effectiveness of granulated dieldrin and heptachlor applied in mid-March on a light snow cover and the same materials as emulsions applied when growth had begun in mid-April. The effectiveness of all treatments was highly significant over the untreated checks. There was no significant difference in the control provided between the emulsifiable concentrates and the granulated forms of the insecticides tested. No significant difference was found in the effectiveness of dieldrin and heptachlor in either the emulsifiable concentrate or in the granulated forms.

Table 1 also shows the comparison between the granulated and emulsifiable concentrate forms of dieldrin and heptachlor when both forms were applied at the early growth stage. The results of all treatments were highly significant when compared with the untreated checks. There was no significant difference in the control provided by the granulated as compared with the emulsifiable concentrate forms of the insecticides tested. There was also found to be no significant difference in the effectiveness of heptachlor and dieldrin in the granulated form. No significant difference was found between the $\frac{1}{4}$ and $\frac{1}{2}$ pound levels of actual insecticide per acre treatments for either dieldrin or heptachlor in the Lawrence County tests.

Due to a late spring in western South Dakota in 1956 the larval damage to the first cutting of hay was just becoming apparent at mowing time. A delay in mowing could have resulted in economic damage. The hay yield as indicated by the samples taken showed no significant differences. Hot weather at mowing time reduced the larval populations in all plots on second cutting alfalfa to a level too low for suitable testing.

The measure of effectiveness of the treatments in the experiments was considered to be the population level of the alfalfa weevil, as measured by sweep samples taken in the individual plots. For the purpose of statistical analysis, the number of weevils per sweep was transformed to logarithms. The analysis of variance technique was applied to the logarithms, and the treatment effects were

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Table 1.—The effect of granulated insecticides compared with emulsions applied for the control of the alfalfa weevil in two counties in South Dakota, 1956.

Insecticide	Pounds of Active Ingredient per Acre	Formulation	Larvae per Sweep at First Cutting	Per Cent Control	Larvae per Sweep at First Cutting	Per Cent Control
			Butte County[a]		*Lawrence County*[b]	
Heptachlor	0.25	(2E) emulsion	1.8	95.2	0.3	96.1
	.25	(2.5%) granulated[c]	8.4	78.1	.1	98.5
	.50	(2.5%) granulated[c]	0.6	98.4	.3	96.1
Dieldrin	.25	(1.5E) emulsion	3.5	90.8	.1	98.8
	.25	(5%) granulated[d]	5.0	87.0	2.2	73.8
	.50	(5%) granulated[d]	5.8	84.8	.3	96.8
Check	—	—	38.4	—	8.4	—

[a] Emulsions applied April 17; granules applied March 16.
[b] Emulsions applied April 14; granules applied April 13.
[c] Particle size 30–60 mesh.
[d] Particle size 30–40 mesh.

separated into individual comparisons as described in Goulden. By this system the significance of each comparison could be statistically determined in terms of the general error. Calculation of a general least significant difference would be unnecessary, since each comparison is already evaluated. The results of the analyses of the experimental data transformed to logarithms are given in table 2.

SUMMARY AND CONCLUSIONS.—Tests conducted in western South Dakota in 1956 showed that the granulated forms of dieldrin and heptachlor provided as effective control of the alfalfa weevil as the emulsifiable concentrated forms of these insecticides. It was also shown that the granulated forms, applied in mid-March on a light snow cover, gave as effective control as the same insecticides applied as sprays at the early-growth stages of the alfalfa in mid-April.

The granulated forms of the two insecticides tested were effective when applied with two types of normally available farm equipment, eliminating the need for an additional investment for equipment for this purpose. The use of this equipment and the granulated insecticides tested were found to be practical at temperatures too low for using field type sprayers. The rancher or farmer may thus control the weevil by applying the insecticides at a time of the year when the time expended will not conflict with the needs of other equally essential early spring field work.

Table 2.—Analysis of variance of alfalfa weevil larvae counts from various treatments transformed to logarithms.

Sources of Variance	d.f.	Mean Squares: Butte County	Mean Squares: Lawrence County
In granules and sprays			
Check×Treatment	1	2.30**	5.40**
Granular×Emulsion	1	0.21 n.s.	0.27 n.s.
Dieldrin Granules×Heptachlor Granules	1	0.03 n.s.	0.92 n.s.
Dieldrin Emulsion×Heptachlor Emulsion	1	0.13 n.s.	0.14 n.s.
Replications	2	0.05 n.s.	0.32 n.s.
Error	8	0.15	0.20
In granules			
Check×Treatment	1	3.16**	5.27**
Dieldrin×Heptachlor	1	0.99 n.s.	0.40 n.s.
Dieldrin: ¼ pound×½ pound	1	0.02 n.s.	0.70 n.s.
Heptachlor: ¼ pound×½ pound	1	2.56**	0.04 n.s.
Replications	2	0.35 n.s.	0.63 n.s.
Error	8	0.21	0.18

** Significant at the 1% level.
n.s.—Non-significant.

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Cabbage Aphid Control[1]

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Heavy infestations of the cabbage, *Brevicoryne brassicae* (L.) are very difficult to control on cabbage, brussel sprouts, and broccoli. In the Weslaco, Texas area, infestations of cabbage aphids usually appear around the middle of January in small local areas scattered throughout a field. Many times these localized infestations are not noticed by growers until the aphid population has built up to destructive numbers. Because of this, many growers usually start their cabbage aphid control program around the middle of January. Research was conducted to find an insecticide which would eliminate or control severe aphid infestations.

Previous work by Wene & White (1952, 1953) showed that applications of malathion, parathion, and methyl parathion resulted in effective aphid control but failed to eliminate the aphid population. Also that the systemic insecticide, demeton, greatly reduced the aphid population, but did not eliminate it.

PROCEDURE.—All the experiments, with the exception of experiment 5, results of which are shown in table 3, were conducted in cabbage fields, which had been planted in rows spaced 40 inches apart. Experiment 5, results of which are shown in table 3, was conducted in a field of brussel sprouts, which also had been planted in rows spaced 40 inches apart. Each of the treated plots referred to in tables 1 and 3 were 0.03 acre in size. The treated plots in the remaining experiments consisted of a single row of cabbage 40 feet in length, with an untreated buffer row between the plots. Each treatment was replicated three times.

The spray treatments were applied with a 3-gallon garden sprayer adapted to apply finished sprays at the rate of 5 gallons per acre. The insecticidal dusts were applied with rotary hand dusters, at the rates shown in table 3.

Effectiveness of the various insecticides was determined by selecting cabbage or brussel sprout plants at random in each plot and counting the aphids on a square-inch area of the heaviest infested portion of the most severely infested leaf on each plant selected. Twenty-five leaves were examined in each of the plots reported in table 1, whereas only 10 leaves were examined in the plots of the remaining experiments.

Table 1.—The residual effectiveness of demeton in controlling cabbage aphids.

DEMETON PER ACRE (LBS.)	WEEKS AFTER APPLICATION			
	1	2	3	4
	Average No. Aphids per Sq. Inch			
0.25	1.1	5.0	6.8	21.3
0.5	0.3	2.0	3.5	11.9
0	5.6	19.9	21.2	28.0
	Per Cent Aphid-free Plants			
0.25	76	44	34	10
0.5	96	57	42	16
0	50	16	8	4

Table 2.—Effectiveness of repeated insecticidal low volume spray applications in reducing cabbage aphid populations.

MATERIAL AND POUNDS PER ACRE	AVERAGE NO. CABBAGE APHIDS PER SQ. IN.				
	Days After 1st Application			Days After 2nd Application	
	1	4	9	1	5
Demeton, 0.25 lb.	2.5	2.7	10.5	0	0.3
Phosdrin, 0.25 lb.	1.3	9.6	32.5	2.0	2.8
Phosdrin, 0.5 lb.	3.0	10.6	36.5	1.0	1.6
Chlorthion, 0.25 lb.	15.6	43.0	29.5	0.6	3.0
Chlorthion, 0.5 lb.	10.0	60.5	23.4	0.4	6.7
Malathion, 1.2 lbs.	1.2	4.3	26.3	0.0	1.2
Untreated	52.8	60.5	48.0	45.5	52.7

Table 3.—Control of cabbage aphids with insecticides applied as low volume sprays or as a dust.

INSECTICIDES	POUNDS PER ACRE	AVERAGE NO. APHIDS PER SQ. IN. AFTER	
		2 Days	4 Days
Experiment 3: Low Volume Sprays			
Malathion	1.2	0	0
Diazinon	0.5	0.4	0
Endrin	0.4	4.1	2.7
Hercules AC-528	0.5	33.7	37.8
Piperonyl butoxide, 60% +pyrethrums, 6%	0.25	37.3	33.5
Untreated	—	45.1	44.7
Experiment 4: Dusts			
Malathion, 5.0%	25	0.2	0
Diazinon, 4.0%	25	0.2	0
Endrin, 1.5%	25	0.3	0
Piperonyl butoxide, 0.9% +pyrethrums, 0.6%	25	19.3	23.4
Untreated	—	33.8	41.5
Experiment 5: Dusts			
Malathion, 7.5%	25	2.4	
	35	1.4	
Phosdrin, 2.0%	25	2.3	
	35	1.3	
Untreated	—	41.9	

DISCUSSION.—At the time the demeton treatments were applied very few aphids were noticed in the field. The data in table 1 show that 0.25 pound of demeton per acre resulted in good protection for a period of 1 week. After the first week the data show that the aphid population increased sufficiently to require a second application. The population records, shown in table 1, indicated that demeton at 0.5 pound per acre was very effective 1 week after the application and still held the aphid population to non-destructive numbers 2 to 3 weeks after the application. In order to be salable cabbage must be "aphid-free" and the data in the table indicate that 43% of the cabbage plants remained infested with aphids 2 weeks after the treatment in the plots treated with 0.5

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Table 4.—Control of cabbage aphids with insecticides applied as low volume sprays.

Material and Pounds/A.	Cabbage Aphids per Sq. In.			
	1 Day		7 Days	
	No.	Per Cent Reduction	No.	Per Cent Reduction
Dipterex, 0.25	24.1	24	32.0	28
Malathion (purified), 0.4	8.3	74	18.0	60
Malathion (purified), 0.8	1.9	94	10.9	76
Malathion, 0.4	6.0	81	6.8	84
Malathion, 0.8	2.0	94	3.1	94
Dow ET-57,[a] 0.25	11.5	64	9.7	78
C. & C. 7744,[b] 0.5	12.7	60	15.8	65
Untreated	31.6	—	44.5	—
L.S.D. at 1% level	10.0		14.3	
5% level	13.9		20.0	

[a] Sampled as Dow ET-14.
[b] (Sevin), N-methyl-1-naphthyl carbamate.

pound per acre of demeton. A week later on these same plots the average population was only 3.5 aphids per square inch, but 58% of the plants were infested. The data illustrate that a single application of demeton does not eliminate a cabbage aphid population, and in order to obtain effective control of the insect with demeton, repeated applications are required (table 2).

Experiment 2 was conducted in a field of cabbage heavily infested with aphids. The data presented in table 2 indicate that 0.25 pound concentration of demeton or Phosdrin, or 1.2 pounds of malathion gave effective cabbage aphid control for a period of 4 days after the applications. Also the data show that Phosdrin lost its effectiveness faster than demeton or malathion. A single application of Chlorthion was not sufficiently effective for commercial control. Nine days after the treatment the aphid population was again large enough to require insecticidal treatment. The second application was more effective than the first, which emphasizes the importance of repeated applications applied at proper intervals.

The data recorded in the remaining experiments, presented in tables 3 and 4, show the toxicity of various insecticides to the cabbage aphid. The data in table 3 indicate that malathion, Diazinon, and endrin, applied either as a spray or a dust gave effective aphid control. A mixture of piperonyl butoxide with pyrethrum failed to give control when applied either as a spray or a dust. Hercules AC-528 failed to control cabbage aphids. Experiment 5, which was conducted in a field of brussel sprouts, showed that 7.5% malathion and 2% Phosdrin dusts were effective in controlling the pest. Increasing the amount of dust from 25 to 35 pounds per acre increased the degree of control very little.

Although the data in table 4 show that the commercial preparation of malathion was more effective than the purified form, the difference was not significant. Dow ET-57 (sampled as Dow ET-14) and Sevin or C. & C. 7744 (N-methyl-1-naphthyl carbamate), show promise in controlling the cabbage aphid and warrant further investigation. Applications of Dipterex failed to control cabbage aphids.

Summary.—Demeton applied at 0.5 pound per acre effectively reduced the cabbage aphid population for a period of 3 weeks, but cabbage aphids were found on 43% of the plants 2 weeks after the treatment.

Phosdrin, malathion and demeton gave excellent cabbage aphid control when applied twice at 9-day intervals.

Promising results in aphid control were also obtained with Diazinon, endrin, Dow ET-57 and C. & C. 7744 (Sevin). The purified formulation of malathion was as toxic as the regular formulation when tested for cabbage aphid control.

Hercules AC-528 and a mixture of piperonyl butoxide with pyrethrums showed very little toxicity to the cabbage aphid.

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Experiments for the Control of the Onion Maggot[1]

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Onions are a major crop in Idaho, and losses caused by the onion maggot (*Hylemya antiqua* (Meig.)) are of local importance each year. In addition to the losses caused to bulb onions, seed yields are often seriously reduced when onion maggots attack the bulbs. The bulb may be destroyed before sending up seedstalks, or the plant may be so weakened that the stalks turn yellow and fall over. Seed that forms on severely injured plants is shriveled and worthless.

Experiments were conducted in southern Idaho during 1951–54 to determine the effectiveness of various insecticides and methods of applying them for the protection of bulb- and seed-onion crops against this maggot. Both crops were started in the spring, the bulb crop from seed and the seed crop from overwintered bulbs. The work was done on the University of Idaho Experiment Station Farm at Kimberley in 1951, on the U. S. Dept. of Agriculture field station grounds at Twin Falls in 1952 and 1953, and in a commercial field at Filer in 1954.

Bulb Onions.—*Materials and Methods.*—Four-row plots planted with a seeder were used except in 1953, when each plot consisted of a single row 3 feet long planted by hand with 100 seeds. The insecticides were ap-

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Table 1.—Stands of bulb onions following soil and seed treatments for onion maggot control in southern Idaho, 1951, 1952, and 1954.

Insecticide	Pounds of Toxicant per Acre	Number of Samples with Maggot Damage	Number of Undamaged Plants per Foot of Row
1951—Soil drenches EC			
Heptachlor 25%	1	1	7.6
Toxaphene 75%	1	11	7.4
Aldrin 25%	1	3	7.3
Chlordane 75%	1	7	7.0
DDT 25%	1	4	6.7
Dieldrin 20%	1	8	6.7
Lindane 20%	½	9	6.7
Check	—	18	5.8
L.S.D. (19:1)			0.8
1952—Soil drenches WP			
Heptachlor 25%	½	19	8.5
Dieldrin 25%	½	15	8.3
Chlordane 40%	1	17	7.6
Aldrin 25%	½	23	7.2
Isodrin 25%	¼	21	6.7
Parathion 25%	¼	43	6.1
Lindane 25%	¼	39	6.0
1952—Seed treatments WP (¼ pound of toxicant per 100 pounds of seed)			
Aldrin 40%	—	9	7.8
Dieldrin 50%	—	4	7.2
Heptachlor 50%	—	9	6.8
Lindane 75%	—	44	5.2
Chlordane 40%	—	50	5.0
Check	—	49	5.1
L.S.D. (19:1)			1.1
1954—Broadcast on soil (3 pounds of toxicant per acre)[a]			
Aldrin 2.5%	—	—	6.7
Heptachlor 1.5%	—	—	5.7
Dieldrin 1.5%	—	—	5.1
Chlordane 5%	—	—	4.5
Check	—	—	4.2
L.S.D. (19:1)			1.2

[a] All samples contained maggot-damaged plants.

plied as soil drenches in 1951 and 1952, as seed treatments in 1952 and 1953, and as broadcast soil treatments in 1954. Soil-drench applications were made with a gear-pump sprayer at 25 gallons per acre. A fan-type nozzle located 5 inches behind the seed drop and 2 inches above the soil surface sprayed both the seed and the sides of the furrow. The seed treatments consisted of mixing 1.1 grams of 75% thiram to 1 pound of seed, stirring in the insecticide, and adding from 1 to 3 grams of a methyl cellulose solution, depending upon the insecticide dosage used. Seeds were stirred until evenly coated, then dried and planted. For broadcast applications, the insecticide was mixed with soil and scattered by hand and the plots were harrowed and planted. Counts were made of undamaged plants in 25 one-foot sections of row except in 1953, when the entire plots were examined.

Results.—All the insecticides applied as soil drenches in 1951 (table 1) increased the plant stand. The heptachlor drench gave the most promising results.

In 1952 (table 1) the heptachlor drench also was the most promising treatment, although dieldrin, chlordane, and aldrin drenches also were significantly better than the check. Heptachlor and dieldrin were significantly better when used as drenches than as seed coatings. However, since from 8 to 33 times as much toxicant was applied in the drenches as in seed coatings, the differences were probably due to the dosages rather than the form of application. Drenches were more difficult to apply, and the small differences in stands failed to justify their use rather than seed coatings. Lindane drenches and seed coatings caused deformed seedlings and thin stands.

In 1954 (table 1) only the aldrin and heptachlor broadcast treatments resulted in stands of plants significantly better than the check. Much maggot damage occurred regardless of the treatment, as the infestation in this experiment was unusually high.

Experiments in 1953 (table 2) proved the value of the seed-coat method of application, since 8 out of 10 check plots were infested but only 13 out of 120 treated plots. It was shown that ½ pound of the insecticide per 100 pounds of seed was as effective as 4 pounds. Moreover, poor stands were produced following treatments of 2 to 4 pounds of aldrin, and other tests not discussed here

Table 2.—Stands of bulb onions following seed treatments with insecticides for onion maggot control at Twin Falls, Idaho, 1953.

Pounds of Toxicant per 100 Pounds of Seed	Number of Plots with Maggot Damage	Average Number of Plants per Plot
Heptachlor 50%, WP		
0.5	3	72
1	1	71
2	2	72
4	1	76
Dieldrin 50%, WP		
0.5	2	72
1	0	71
2	0	70
4	1	65
Aldrin 40%, WP		
0.5	1	65
1	1	71
2	0	61
4	1	62
Check	8	41
L.S.D. (19:1)	—	12

Table 3.—Onion maggot adults caught in sticky traps during biweekly periods at Twin Falls, Idaho, 1952–54.

Period	1952	1953	1954
April 9–22	0	0	0
April 23–May 6	9	3	0
May 7–20	14	5	3
May 21–June 3	13	16	18
June 4–17	40	24	53
June 18–July 1	163	64	40
July 2–15	115	34	21
July 16–29	46	11	0
July 30–August 12	74	5	1
August 13–26	18	1	0

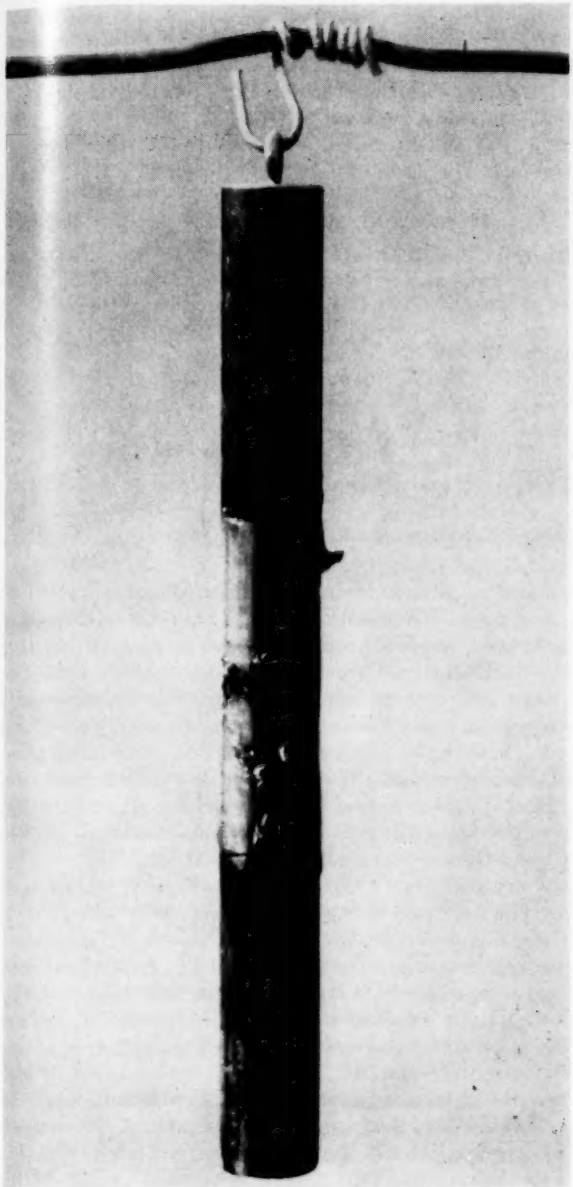

FIG. 1.—Sticky trap made of wood, $\frac{5}{8}$ inch in diameter and 6 inches long. The middle section is coated with an adhesive, leaving the uncoated ends to take hold of while examining under binoculars.

corroborated the results, indicating that heavy dosages were deleterious to germination. Seed coatings are inexpensive and easy to apply, and they give valuable protection under moderate infestations.

SEED ONIONS.—*Materials and Methods.*—The bulbs were set close together in rows 30 inches apart. Treatments were replicated seven times in 1952 and six in 1953 and 1954. Yields were generally used in comparing results of the tests, but in 1952 the numbers of onion maggot pupae found in the soil at harvest time also were used.

Sticky traps (fig. 1) were used to determine the time of adult emergence (table 3). Emergence usually began late in April and reached a peak the latter part of June.

In 1952 applications were made on May 22, June 16, and 21 before the peak flight occurred. A knapsack sprayer was used, and the nozzle was held low in order to wet only the base of the plants and a strip of soil 6 inches wide in the row. One pound of toxicant was applied in 25 gallons of spray per acre, except for lindane which was used at $\frac{1}{2}$ pound.

In 1953 and 1954 the bulb-soaking method used by Doucette (1953) for control of the narcissus bulb fly was tried. Weighed lots of bulbs were either soaked for 10 minutes or dipped for 5 seconds in emulsions containing $\frac{3}{4}$ pound of toxicant in 50 gallons of water. The temperature of the bath was between 65° and 70° F. After treatment the bulbs were planted in single-row plots at the ends of commercial fields.

In other treatments in 1954 dusts mixed with soil were applied in the furrows before setting out the bulbs. Three pounds of toxicant per acre was used, the application being confined to a strip about 5 inches wide in each row.

Results (table 4).—Dieldrin, heptachlor, DDT, lindane, and aldrin, applied in sprays to growing plants prior to the peak of adult onion maggot emergence all gave significantly higher yields than the check. The yield of seed was increased 225 pounds per acre where the dieldrin spray had been applied. The spray method of application, however, might not be feasible in large fields because of wheel damage from the heavy equipment. It seems best adapted to small fields and gardens where knapsack sprayers can be used.

Table 4.—Onion seed yields following treatment for onion maggot control in southern Idaho, 1952–54.

INSECTICIDE	PUPAE PER FOOT OF ROW AFTER HARVEST	YIELD OF SEED, POUNDS PER ACRE
1952—Spray treatments, EC		
Dieldrin 20%	0.8	697
Heptachlor 25%	1.6	697
DDT 25%	2.6	612
Lindane 20%	2.8	588
Aldrin 25%	1.8	578
Chlordane 75%	2.1	549
Toxaphene 75%	3.1	481
Check	3.3	442
L.S.D. (19:1)	—	116
1953—Bulbs soaked 10 minutes, EC		
Chlordane 75%	—	728
Heptachlor 25%	—	698
Aldrin 25%	—	642
Dieldrin 20%	—	573
Check	—	460
L.S.D. (19:1)	—	157
1954—Bulbs soaked 10 minutes, EC		
Chlordane 75%	—	421
1954—Bulbs dipped 5 seconds, EC		
Chlordane 75%	—	364
Heptachlor 25%	—	326
1954—Dusts applied in furrows		
Aldrin 2.5%	—	397
Chlordane 5%	—	333
Check	—	225
L.S.D. (19:1)	—	109

The bulb-soak method also gave significant increases in yields of seed where the bulbs had been treated with aldrin, chlordane, dieldrin, or heptachlor. Dipping the bulbs in chlordane or heptachlor emulsions also improved the yields of seed, although in the case of chlordane dipping was inferior to soaking. Applications of aldrin and dieldrin in the furrows also gave increases in yields of seed.

SUMMARY.—Aldrin, chlordane, DDT, dieldrin, heptachlor, isodrin, lindane, parathion, and toxaphene were tested as dusts, dips, soaks, sprays, and drenches for control of the onion maggot (*Hylemya antiqua* (Meig.)) on onions grown for bulbs and seed in south-central Idaho from 1951 to 1954. Improvements in yields of seed or the number of undamaged bulbs were noted with several insecticides and methods of application. Coating the seed with aldrin, dieldrin, or heptachlor prior to planting gave best control on bulb crops. Soaking the bulbs in chlordane, heptachlor, or aldrin emulsions gave the best control on seed crops.

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Incidence of Polyhedrosis of Gypsy-Moth Larvae and the Influence of Relative Humidity[1]

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Since the gypsy moth, *Porthetria dispar* (L.), has developed into a serious economic problem in New England, measures have been sought for natural control of this forest pest. More than 40 years ago, Glaser (1915, 1918) and Chapman & Glaser (1916) recognized that diseases of the gypsy-moth larvae might be useful as natural control measures, and initiated an investigation of this possibility. Since then, little has been published concerning investigations of the polyhedrosis in New England.

Within recent years, however, success has been reported concerning results of experiments utilizing viruses for control of other insects of economic importance. Among these investigations a polyhedrosis has been described in the alfalfa caterpillar (Steinhaus 1948) and attempts to utilize it for control purposes have been reported (Thompson & Steinhaus 1950). There are descriptions of dissemination and propagation of viruses affecting the European spruce sawfly (Bird 1950, 1954, 1955, Dowden & Girth 1953, Franz & Niklas 1954). Recently, Tanada (1953) described the characteristics of a granulosis virus of the imported cabbageworm. Other investigators have reported experimental dissemination of a polyhedrosis virus for control of the Great Basin tent caterpillar (Clark & Thompson 1954, Clark 1955, 1956, Clark & Reiner 1956).

Although these studies have developed rapidly within recent years, the status of the polyhedrosis virus disease of the gypsy-moth larvae along with the similar polyhedrosis disease of the silkworm caterpillar, has remained in a somewhat controversial stage of evaluation on the basis of laboratory experimental results. This subject has been reviewed in detail by Steinhaus (1949) and Bergold (1953). The laboratory investigations concerning the infectiousness of the polyhedrosis virus apparently were complicated by the presence of "latent" virus in the caterpillar populations. Vago (1951) described this condition in the silkworm and reported methods of "activating" the "latent" infection by subjecting larvae to a variety of adverse conditions. Yamafugi (1951) described a similar series of experiments with the silkworm in which chemical treatment of larvae with hydroxylamine and potassium nitrite resulted in increased mortality due to virus disease. While these studies have been followed in laboratory studies, severe epizoötics continue to occur in the field among late instar larval populations. However, recent attempts to initiate an epizoötic by spraying a suspension of polyhedral bodies over an area of woodland infested with the gypsy moth (Dowden 1956) failed to induce any detectable increase in the low incidence of polyhedrosis in the larval population. To investigate this problem laboratory and field studies were initiated in Connecticut by the author in 1953.

EXPERIMENTAL OBSERVATIONS.—Gypsy moth populations and polyhedrosis epizoötics were observed in Connecticut during 1953, 1954, and 1955. Egg masses were collected in a variety of ecological situations each year from sites which were located in 10 widely separated areas. These included old established infestation areas of low population where no disease had yet appeared among the caterpillar population, old infestation areas of high population where epizoötics had occurred, and newly infested areas of high and low populations. From these areas a total of 538 egg masses were collected, stored in separate envelopes in refrigeration, and reared in the laboratory as individual colonies. Each egg mass was placed in a sterile 1-quart wide-mouthed glass jar covered with a muslin cloth which was securely held in place with rubber bands. Particular precautions were taken to prevent cross-contamination. Egg masses, from 1 to 20 at a time, were maintained separately throughout the late winter and spring following the season in which they were collected. Larvae from them were reared in separate colonies and were fed washed fresh lettuce leaves in the winter months and washed oak leaves during late spring months. Each colony was maintained until presence of polyhedrosis was confirmed by isolation and concentration of polyhedral bodies from diseased larvae, and then the colony was discontinued. Rearing conditions were utilized which provided an *excess* of humidity for larvae.

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This was partly supplied from wet lettuce leaves in the jars in excess of the amounts utilized for food. Additional humidity was supplied from cotton pads saturated with water and placed on the covering of the jars. During the winter of 1953–54, 190 separate colonies were reared. Polyhedrosis appeared in each colony. In the winter of 1954–55 an additional 230 colonies were reared. Polyhedrosis appeared in each colony. During the winter of 1955–56, another 118 colonies were reared and polyhedrosis again appeared in each colony. Thus, a total of 538 colonies were reared and in each one polyhedrosis appeared, regardless of the source and type of infestation area from which the egg-mass originated.

To determine the effects of chemical "stress" upon gypsy-moth larvae, hydroxylamine hydrochloride (a strong reducer) was applied to food in a series of experiments. Fresh oak leaves were dipped in solutions consisting of 1 Normal, 1/10 Normal and 1/100 Normal concentrations. The solution was allowed to dry on the surface and the leaves were then placed in 1-quart glass jars which contained 20 larvae each. The larvae were apparently healthy third-instars from stock colonies. The experiment was replicated three times. Untreated controls in each experiment were fed washed fresh oak leaves, and daily mortality was recorded. The procedure was then repeated in the same manner, except that potassium nitrite solutions were used.

In these tests of chemical "stressors" administered orally on the food of larvae, considerably greater mortality due to polyhedrosis occurred among groups of larvae which fed on leaves treated with 1 N hydroxylamine solution than in controls. In these test groups, 38.3% of 60 larvae died from polyhedrosis. However, an additional 30% died from undetermined causes. In control groups under the same conditions, mortality due to polyhedrosis appeared in 16.6% of 60 larvae. In the controls there were an additional four deaths from undetermined causes. In tests of 1/10 N hydroxylamine 21.6% of 60 larvae died of polyhedrosis, with 12 undetermined deaths. In tests of 1/100 N hydroxylamine, 23.3% of 60 larvae died of polyhedrosis and five larvae died of undetermined causes.

Larval mortality in tests utilizing potassium nitrite administered via the larval food was very similar to that detailed above. In tests of the 1/10 N concentration, a 25% incidence of polyhedrosis occurred as compared with the 10% which resulted among the controls. However, unusual mortality other than that due to polyhedrosis occurred in tests utilizing 1 N potassium nitrite. Leaves treated with this concentration of test chemical were subjected to severe "leaf-burn," and they turned brown while the larvae were still feeding.

During an investigation of conditions of an epizoötic in the field, an area in Bethany, Connecticut, (in which a very high population of gypsy-moth larvae occurred in mixed hardwood and coniferous woodland) was kept under observation from the time of gypsy moth hatching in the spring until the onset of a severe epizoötic of polyhedrosis occurred among the larvae shortly after mid-July. Laboratory-reared populations were maintained for control observations. These consisted of 100 second instar and 100 third instar larvae which were maintained on washed fresh black oak leaves. For comparison with field counts, daily mortality in these populations was recorded during the period from June 14 to July 9, 1956. Daily relative humidity observations were recorded both in the laboratory and in the field. The relative humidity in the field and daily mortality due to polyhedrosis were compared as shown in figure 1.

Each day, from June 14 to July 10, 1956 (with exception of several week-end days indicated in fig. 1) the incidence of newly diseased larvae per 1000 was recorded. During a 21-day period from June 14th to July 5th, the incidence of diseased larvae in the population was low (fig. 1). During this period of time, unusually dry weather conditions existed as shown by the daily minimum relative humidity (r. h.) recorded in figure 1. However, precipitation occurred on July 4th, and during the next 5 successive days high r. h. prevailed. Following this sharp rise in r. h. the daily incidence of diseased larvae increased. From three per 1,000 on July 5th, the mortality increased with each successive day of high r. h. to 58 per 1,000 by July 10th. Counts of larval mortality were discontinued at this time because a large proportion of the larval population was undergoing pupation.

The same trend in the incidence of polyhedrosis was observed in populations of younger larvae in the laboratory. Among 200 third- and fourth-instar larvae which were observed from June 14th to July 5th, eight per cent mortality due to polyhedrosis was observed. During this period the r. h. in the laboratory averaged 10% higher than that recorded in the field, and it followed the same pattern of increase to 98% between July 4th and 10th. The incidence of polyhedrosis in the laboratory during this period of high r. h. rose from 8 to 42%.

Observation of this phenomenon, both in the field and in the laboratory, led to preliminary study of the relationship of the incidence of polyhedrosis and high atmospheric r. h. in the environment of the gypsy-moth larvae. A series of laboratory experiments were conducted to further investigate the effect of relative humidity on the appearance of polyhedrosis. Eggs from one egg-mass were separated and washed in 10% formalin solution for 1 hour. They were then washed with a dilute solution of detergent cleaner (Alconox), rinsed in tap water three times and allowed to dry. Five eggs were placed in each of 30 sterile vials (15-dram), and when hatched, the young larvae were fed bits of lettuce leaves through the second instar and then transferred to ½-pint sterile glass jars which were covered with nylon bobbinet. Two series of jars were prepared, each of which consisted of jars containing 1, 2, 3, 4, 5 and 10 larvae. One series was maintained in a laboratory room in which r. h. was controlled at $60 \pm 5\%$ at 74 ± 2 degrees F. The other series was maintained in a laboratory room in which the r. h. was $30 \pm 10\%$ at 74 ± 5 degrees F. Larvae in each series were fed bits of washed fresh lettuce leaves, and daily mortality due to polyhedrosis was recorded. Each series was repeated and the results recorded.

In the two different conditions of r. h., there resulted a very different incidence of mortality due to polyhedrosis. Larvae maintained in the low r. h. ($30 \pm 10\%$) remained healthy and ate vigorously. On the contrary, those maintained in the room with high r. h. ($60 \pm 5\%$) appeared sluggish in migrating activity, fed lightly and rapidly succumbed to the onset of polyhedrosis. Under these conditions, the number of larvae crowded into the

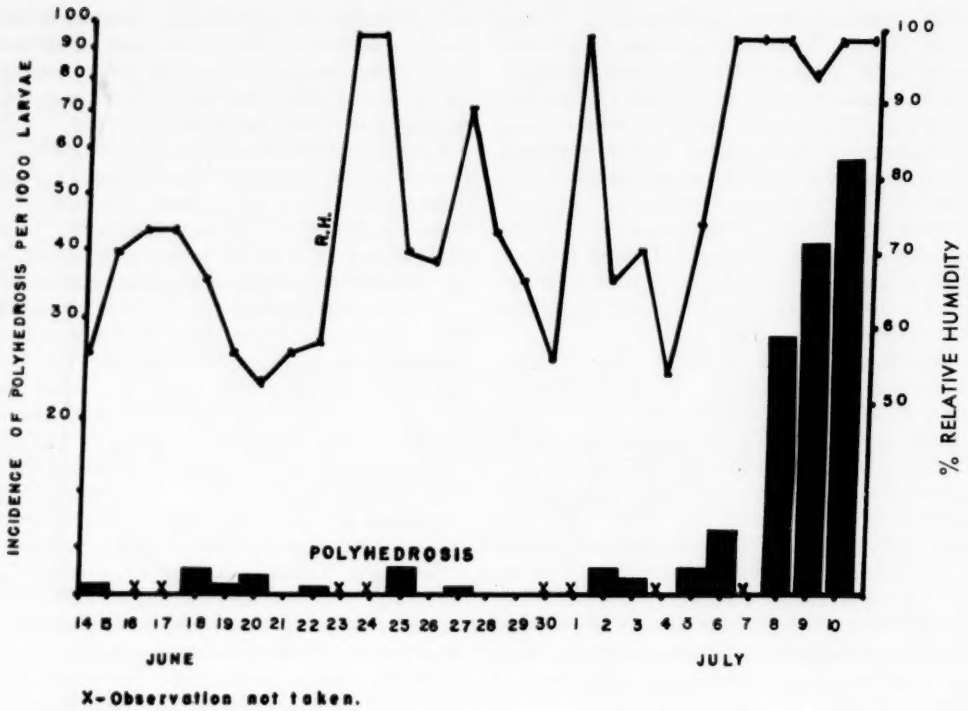
INCIDENCE OF POLYHEDROSIS PER 1000 LARVAE
% RELATIVE HUMIDITY
R.H.
POLYHEDROSIS
14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 1 2 3 4 5 6 7 8 9 10
JUNE
JULY
X-Observation not taken.

FIG. 1.—Daily minimum per cent relative humidity, June 14, to July 10, 1956, and the incidence of polyhedrosis per 1000 gypsy-moth larvae at Bethany, Connecticut.

jars was not effective as a "stress" factor, inasmuch as polyhedrosis appeared in jars containing 1, 2 and 3 larvae as soon as it appeared in jars which contained 10 larvae.

In all results where larval death is indicated "due to polyhedrosis," polyhedral bodies were isolated from each dead larva. When polyhedral bodies could not be isolated, death was indicated "due to undetermined causes." The isolated polyhedral bodies were subjected to microscopic examination and compared with a sample of polyhedral bodies which was generously supplied by Dr. G. H. Bergold in 1953.

DISCUSSION.—Bergold (1953) pointed out that transmission of polyhedrosis is the most controversial subject in the whole field of insect viruses. From the rapid build-up of an epizoötic and the destruction of mass populations in nature it was supposed that the virus was highly infectious. Since it is an established fact that polyhedral disease is contracted by contaminated food (Bergold 1953), outbreaks of the disease in laboratory stock colonies were generally attributed to non-sterile rearing conditions and contaminated food. This would have been plausible if the infectiousness of the virus was found to be high. However, the appearance of disease in colonies protected by sterile precautions, and the failure in attempts to artificially disseminate the virus for control purposes indicated the low infectiousness, the "latency," and the possibility of transovarian passage of the virus. Bergold (1953) has summarized results of laboratory tests which indicated the low infectiousness of air-dried polyhedral bodies fed to *Porthetria dispar* larvae, and the "latency" of the virus in apparently healthy larvae. Therefore, it is reasonable to postulate that vast epizoötics among gypsy-moth larval populations are the results of activation of a "latent" disease by external environmental factors which serve as "stresses" on the host-larvae in a fashion similar to those factors described by Vago (1951) which activated "latent" polyhedral disease in silkworm larvae. For evidence to support this hypothesis it seemed advisable to include epidemiologic study in the field with laboratory investigation. Inasmuch as all the gypsy moth infestation in New England appeared to have resulted from the original introduction of the pest into Massachusetts in 1868 or 1869 (Forbush & Fernald 1896), a "latent" infection transmitted from generation to generation should theoretically have been dispersed widely throughout the natural population. Results of this study seem to indicate that this has happened. Regardless of the origin of egg masses collected in Connecticut among 10 widely separated sites including a variety of ecologic situations, polyhedrosis appeared in each of 538 colonies which were individually reared over a period of 3 years. With this evidence of virus infection present throughout the larval population, it was reasoned that localized destructive epizoötics were due to an "activation" of the virus by environmental factors. If so, these factors, and possibly other artificial "stressors" (such as orally administered hydroxylamine and potassium nitrite used by Yamafugi (1951) in work with the silkworm) should be effective in increasing the incidence of mortality due to polyhedrosis in the laboratory. Tests with chemical "stressors" gave results which were somewhat encouraging, but the administration of adverse food substances, overcrowding, and irradiation (Wallis 1954), were not as effective as ex-

pected—except under conditions of extremely high atmospheric r. h. This, along with the observation of an increased mortality rate in the gypsy-moth larval population at Bethany, Conn., corresponding with the change from low to high atmospheric r. h. (fig. 1), led to the belief that atmospheric r. h. was a critical factor in the epizoötiology of polyhedrosis. Laboratory tests with larvae under high and low r. h. in the laboratory supported this hypothesis.

The stimulation of feeding and larval vigor which occurred under low r. h. conditions may in part explain the high degree of forest defoliation observed in certain localities. High trap-rock ridges and sandy hill-top locations in gypsy moth infestation areas have long been known to be susceptible to heavy defoliation from larval population densities which caused only slight defoliation of lowland forest sites in the same area. While many other factors may be involved in the ecology which affect larval activity of gypsy moths (Bess *et al.* 1947, Bess 1945, Behre *et al.* 1936, Burgess & Baker 1948, Crossman 1925, Goldschmidt 1933, Mosher 1915, Summers 1922) it is suggested that r. h. has an important influence on the larval population by increasing the incidence of polyhedrosis at high percentages and by stimulating larval feeding and vigor at low percentages.

SUMMARY.—The incidence of polyhedrosis in gypsy-moth larvae, *Porthetria dispar* (L.), was studied in Connecticut from 1953 to 1956 inclusive. During that time polyhedrosis appeared in 538 colonies from individual egg masses which were collected in 10 geographically separated localities in a wide variety of ecologic conditions. In laboratory tests, orally administered chemical "stressors" (hydroxylamine and potassium nitrite) resulted in only slight increase in incidence of polyhedrosis. During field studies of an epizoötic at Bethany, Connecticut in 1956, and concurrent study of laboratory larval populations, little polyhedrosis was observed in a 2-week period of low relative humidity. However, corresponding with a sharp rise in atmospheric relative humidity, the incidence of polyhedrosis increased. Laboratory tests for larval survival resulted in no incidence of polyhedrosis in low relative humidity conditions and a high incidence in high relative humidity conditions.

It was suggested that relative humidity may be a factor of considerable importance in the epizoötiology of the polyhedrosis of the gypsy moth.

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Further Biological Studies on the Western Cherry Fruit Fly[1]

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Several phases of the life history of the western cherry fruit fly, *Rhagoletis cingulata indifferens* Curran (Blanc & Keifer 1955), were studied after Frick *et al.* (1954) published on the bionomics of the cherry fruit flies in eastern Washington. Because of the pressure of other projects, biological studies have been terminated and it is felt that these data will supplement that previously published.

SPECIES INVOLVED.—Blanc & Keifer (1955) showed that differences exist, particularly in the thoracic spiracles of the larva, between the eastern and western populations of the cherry fruit fly. However, these workers lacked specimens from the Yakima Valley, an apparently introduced population. The species was first reported in the city of Toppenish and seems to have spread outward in all directions into the cherry growing districts of the valley (Eide 1945). Toppenish is at least 20 miles from the native hosts of the cherry fruit fly. The primary native host is the bitter cherry, *Prunus emarginata* (Dougl.), while the western choke cherry, *P. virginiana* var. *demissa* (Nutt.), is attacked to a lesser extent.

Blanc & Keifer (1955) illustrated typical and atypical color patterns on the wings of both the eastern and western subspecies. Frick *et al.* (1954) included photographs of the wings of three different flies collected in the Yakima Valley. Two are typical in having the distal extension of the apical costal band united (front cover and fig. 34a) and one is atypical in that the distal extension is separate (fig. 1). Because the differences in the adults of the eastern and western subspecies are not decisive, the numbers of papillae on the thoracic spiracles of the larvae were counted. Larvae from both bitter and choke cherries are included because Blanc and Keifer lacked Washington specimens from those hosts. Based upon the numbers of papillae on the thoracic spiracles, all of the populations studied from central Washington belong to the western subspecies, *indifferens* (table 1). Larvae of the eastern subspecies bear from 21 to 31 papillae per spiracle (Blanc & Keifer 1955). That a single population exists, at least in central Washington, was further supported by the work of Simkover (1953). He showed that adults from populations from native and cultivated species of cherries would

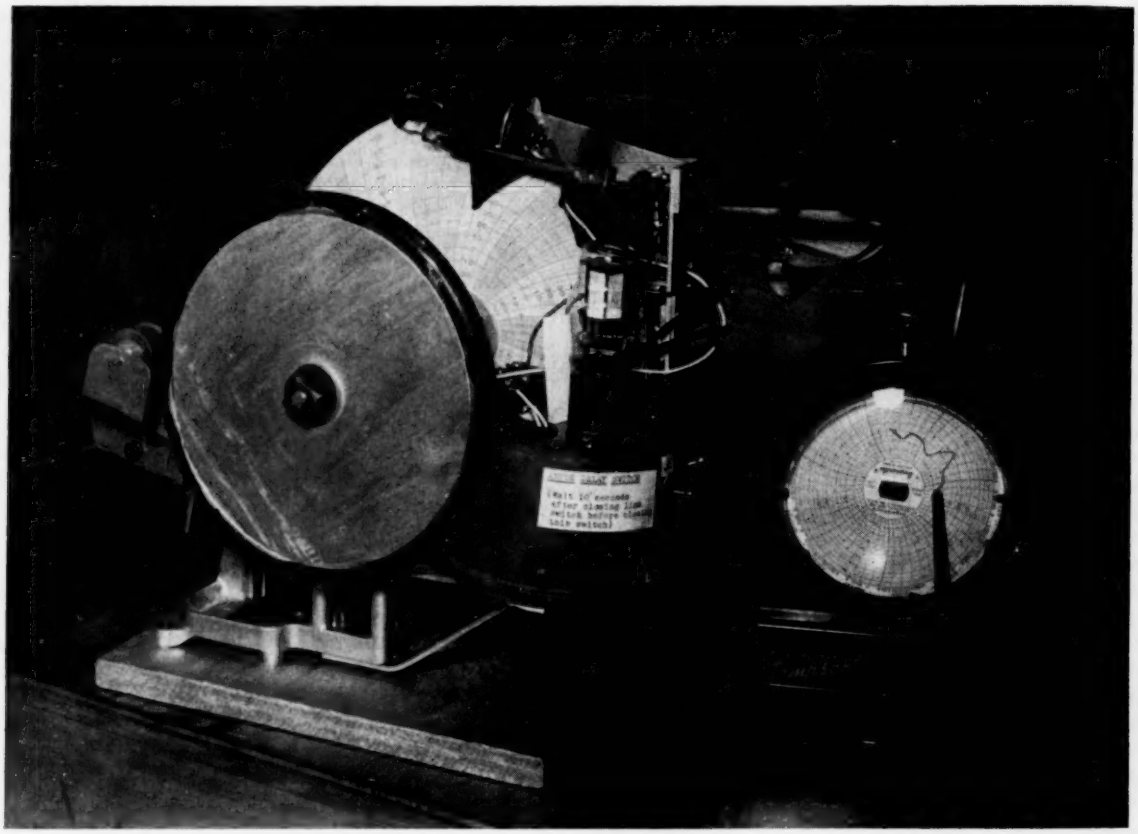

FIG. 1.—Variable temperature control unit used in cabinets to simulate orchard air temperatures. Temperature recorder at right shows actual temperatures maintained in the cabinet.

[1] Scientific Paper No. 1592, Washington Agricultural Experiment Stations, Pullman. Project No. 791. Accepted for publication March 26, 1957.

[2] The writer wishes to thank Mr. Roy E. Bry for carrying out many of these studies and Mr. William B. Rasmussen for designing and building the variable temperature control units.

Table 1.—Numbers of papillae on the thoracic spiracles of western cherry fruit fly larvae from several central Washington populations.

Location	Species of *Prunus*	Total No. of Spiracles	No. of Papillae per Thoracic Spiracle		
			Minimum	Maximum	Mean
Prosser, Benton Co.					
June 28, 1949	*cerasus*	29	10	13	12
July 10, 1954	*cerasus*	30	10	16	14
July 20, 1954	*emarginata*[a]	40	11	16	14
Wenatchee, Chelan Co.					
July 3, 1950	*cerasus*	10	10	15	12
July 27, 1954	*emarginata*	18	11	16	13
White Salmon, Klickitat Co.					
July 25, 1951	*emarginata*	6	9	16	13
July 25, 1951	*virginiana* var. *demissa*	20	12	17	14

[a] In a planting of cherry species and varieties at the Irrigation Experiment Station.

freely interbreed and that there were no differences in either the posterior spiracles of the larvae or the width of the ejaculatory apodeme of the males.

TEMPERATURE REQUIREMENTS OF THE PUPAE.—Pupae that had been refrigerated 6 to 7 months were able to withstand temperatures down to 8° F.[3] for 1 hour when the temperature was gradually lowered from 35° to 8° in 24 hours. A temperature of −20° for several hours killed all pupae.

Refrigeration (32° to 40°) for 6 to 7 months broke the diapause and resulted in about 86% emergence when the pupae were subsequently held at 80° (table 2). However, holding the pupae from 493 to 521 days resulted in heavy mortality. About 65% were dead soon after removal from refrigeration. Nearly 23% more died during a 46-day developmental period at 70°. That 80° was more severe on the pupae is shown by the fact that about 92% of the pupae were dead after 46 days.

Pupae were held at several constant temperatures in cabinets previously described by Frick *et al.* (1954). The pupae were counted out into lots of 200 and each lot placed into 125 ml. of soil in crystallization dishes. At each temperature the soil was moistened periodically with water held in the same cabinet. After 45 days, the puparia were opened and the condition of the pupae noted (table 3).

No development occurred at 50° and about one-third of the pupae developed to some extent at 60°. At 70° most pupae were in the pre-adult stage, but no flies had emerged during the 45-day period. At 80°, conditions were optimum while 90° was lethal to 51.5% of the pupae. Another 25% failed to develop. A high percentage (62%) of the few adults that did emerge were deformed by having undeveloped, twisted wings.

A study was made to determine the effects of placing pupae at 90° from 80° or at 80° from 90° at 7-day intervals throughout the developmental period. The results of holding pupae at 80° and removing them to 90° are given in table 4. It is noteworthy that at 27 days at 80°, when adult emergence had started 3 days earlier, there was a reduction in flies and an increase in deformed flies and pupae dying after developing. These factors became more pronounced as the time at 80° was decreased because the pupae were increasingly less developed before transfer.

On the other hand, holding pupae at 90° is not detrimental up to 21 days but is detrimental beyond 27 days (table 4). During the first 3 weeks pupae were removed to 80° before much development had taken place. At 80° they continued to develop normally. At 27 days and beyond at 90°, development had started and the pupae were injured so that few normal flies emerged. There were high percentages of arrested development after more than 21

Table 2.—Development and mortality of pupae of the western cherry fruit fly put into refrigeration on August 12, 1955 and removed during February and March, 1956, or during late November and December, 1956, and January, 1957. Prosser, Washington.

Total No. of Pupae	1434	650	1530	1506
Refrigeration period (Days)	195–228	493–521	505–512	514–521
Out of refrigeration at 80° F. (days)	60[a]	1–8	46[b]	46
Per cent:				
Adults	86.4	0	1.6	1.2
Developing pupae	0.5	0	3.4	1.2
Undeveloped pupae	6.4	35.0	7.2	5.7
Dead pupae, before developing	6.7	65.0	86.4	90.6
Dead pupae, after developing	0	0	1.4	1.3

[a] 85.8% of the flies had emerged after 46 days.
[b] These pupae held at 70° F. after removal from refrigeration.

Table 3.—Development and mortality of lots of 200 pupae of the western cherry fruit fly held at different temperatures for 45 days during June and July, 1955. Prosser, Washington.

	Temperature, ° F.				
	50	60	70	80	90
Per cent:					
Adults	0	0	0	92.0	21.5
adults deformed	0	0	0	5.0	62.0
developing pupae	0	33.0	86.5	1.5	2.0
undeveloped pupae	87.0	60.0	7.0	2.0	25.0
dead pupae, before developing	13.0	7.0	0	4.5	12.0
dead pupae, after developing	0	0	6.5	0	39.5

Table 4.—Development and mortality of pupae of the western cherry fruit fly held at 80° or 90° F. for varying periods and then transferred to 90° or 80° F., respectively, during June and July, 1955. Prosser, Washington.

Held at 80° F. and then transferred to 90° F.						
No. Days at 80° F.	51	35	27	21	14	7
No. Days After Transfer to 90° F.	0	16	24	30	37	44
Per cent:						
adults	89.5	85.5	66.5	56.5	44.5	20.5
adults deformed	6.4	20.0	24.0	38.0	54.0	75.0
developing pupae	0	0	0	0	0	2.5
undeveloped pupae	1.0	3.5	2.0	4.0	17.5	48.0
dead pupae, before developing	9.0	8.5	7.0	8.5	7.5	8.0
dead pupae, after developing	0.5	2.5	24.5	31.0	30.5	21.0
Held at 90° F. and then transferred to 80° F.						
No. Days at 90° F.	51	35	27	21	14	7
No. Days After Transfer to 80° F.	0	16	24	30	37	44
Per cent:						
adults	1.0	13.0	28.5	84.5	86.0	91.5
adults deformed	0	39.0	21.0	5.0	5.0	3.3
developing pupae	6.5	56.5	54.0	3.5	0	0
undeveloped pupae	46.5	8.5	2.5	5.0	1.0	1.0
dead pupae, before developing	13.0	10.0	6.5	4.5	9.0	7.0
dead pupae, after developing	33.0	12.0	8.5	2.5	4.0	0.5

[3] All temperatures are given in ° F.

Table 5.—Relationship of adult emergence to harvest, fruit infestation, crop size, and prevailing weather in an unsprayed Bing cherry orchard. Prosser, Washington.

No. of Weeks Before Harvest	Percentage of Seasonal Totals of Cherry Fruit Flies Caught in Bait Traps Over an 8-Year Period							
	1949	1950	1951	1952	1953	1954	1955	1956
4	0	3	0.2	0.0	0.02	0.02	0.2	2
3	2	7	0.6	0.3	0.02	0.3	3	14
2	8	24	6	1	0.2	1	20	27
1	33	64	29	2	2	2	49	44
Harvest	59	80	62	14	8	13	64	54
No. days from first emergence to harvest	26	39	40	25	33	36	33	30
Fruit infestation, maggots per 100 fruits	25	47	35	1	13	15	52	85
Estimated size of crop[a]	H	L	H	M	VL	L	M	VL
Mean temperature, from first adult emergence to harvest	63.9[b]	62.1	62.7	63.4[b]	57.3	60.0	64.4[c]	64.9[c]

[a] H, heavy; L, light; M, moderate; VL, very light.
[b] Early season relatively cool, warm just before harvest.
[c] Early season relatively warm, cool just before harvest.

days of 90°. Constant temperatures of 90° or higher are very detrimental to cherry fruit fly pupae after development has started to take place or during the developmental period.

ADULT EMERGENCE AND ACTIVITY UNDER FIELD TEMPERATURES IN ORCHARD AND LABORATORY.—During the last eight seasons, the extent of damage caused by the cherry fruit fly has varied greatly in an unsprayed orchard (table 5). This variation has been due primarily to the temperatures prevailing during each season and to some extent to the size of the crop. That the fly could make such a rapid comeback after the cool early-season weather of 1952, 1953, and 1954 indicates that when early-season temperatures are high, the cherry fruit fly can quickly become a menace in the Yakima Valley. The time of first emergence and the rapidity of emergence depend directly upon the prevailing temperatures. The 1955 and 1956 seasons were slightly warmer than in 1949 or 1952. The highest temperatures came during May in 1955 and 1956 while June was the hottest period in 1949 and 1952. The early extremely warm weather in 1956 resulted in the most rapid early-season adult emergence of the 8 years.

Laboratory studies were conducted to compare the effects of a warm and a cool season upon the adults. When these tests were made, the 1949 season was the warmest and 1953 was the coolest. To simulate orchard air temperatures in the temperature cabinets, two photoelectric cam-follower variable temperature control units were built for use in the cabinets (fig. 1). The units were designed so that the photoelectric cell controlled a single pole double throw relay. When the cell was not actuated, the relay was off and the cold was turned on. When the cell was actuated, the relay was on and the heat units were started. Weekly temperature charts that followed the hourly temperatures of the 1949 and 1953 seasons were made. These were glued to masonite or hardboard. Then the masonite was cut out in the patterns of the charts. The photoelectric cell rode on the chart as the large wheel made one revolution in 7 days.

In a preliminary test, the week of June 5 to 11 was chosen because of the great difference in mean temperatures between 1949 and 1953. The 1949 mean was 10° higher than that of 1953 (69.8° compared with 59.7°). The mean maximum was 15.7° higher (89.4° compared with 73.7°), and the mean minimum 4.6° higher (50.3° compared with 45.7°). Eight females and eight males were caged in each cabinet. The females held at 1949 temperatures each averaged 2.6 full-sized and 8.5 half-sized ova in the ovaries. No development took place at the 1953 temperatures.

In a more elaborate test, the 3 weeks following May 15 were chosen to determine ovarial development before harvest for the 1949 and 1953 seasons. For these 3 weeks, the mean temperature was 6.2° higher in 1949, the mean maximum 9° higher, and the mean minimum 3.3° higher. Ripe, fresh cherries were provided daily for oviposition. Under the warmer 1949 temperatures, the females began ovipositing in 10 days and averaged 19.5 eggs each. The females at 1953 temperatures laid no eggs and the ovaries averaged only 4.2% of the fully developed ova found in the 1949 females (table 6). These tests provided quantitative data that show why cherries were more heavily infested in 1949 than in 1953.

EFFECT OF HUMIDITY AT 90° UPON THE ADULTS.—During the temperature studies, it was noted that at 90°, high relative humidities appeared detrimental to the flies. Further tests confirmed this observation. At relative humidities above 75% both longevity and fecundity were

Table 6.—Comparison of ovarial development in females held at the prevailing air temperatures of the three weeks from May 15 to June 4, 1949 and 1953. Prosser, Washington.

Year	1949	1953
No. of Flies	19 ♀ ♀, 20 ♂ ♂	22 ♀ ♀, 20 ♂ ♂
Weekly Mean Air Temperature[a]		
May 15–21	60.8 (74.1–47.4)	56.6 (70.7–42.6)
May 22–28	63.5 (80.9–46.1)	53.5 (64.3–42.6)
May 29–June 4	60.7 (76.4–44.9)	56.2 (69.1–43.3)
Minimum preoviposition period (Days)	10	—
Average no. of eggs oviposited per female	19.5	0
Average no. of ova in ovaries after 3 weeks:		
fully developed	12	0.5
half developed	20	7

[a] Mean maximum and mean minimum given in parentheses.

Table 7.—Effect of humidity upon longevity and fecundity of adult cherry fruit flies held at 90° F. during June and July. Prosser, Washington.

	PER CENT RELATIVE HUMIDITY			
	10–20	40–50	50–60	75–82
NO. OF FLIES	27♀♀, 27♂♂	10♀♀, 10♂♂	31♀♀, 35♂♂	15♀♀, 15♂♂
YEAR TEST CONDUCTED	1955	1953	1955	1954
Days from Emergence to:				
50% mortality	36	47	33	12
100% mortality	73	73	56	20
Copulation:				
first observed (days)	8	3	8	5
last observed (days)	22	19	21	9
Minimum preoviposition period (days)	7	5	8	5
Emergence to last oviposition (days)	49	41	46	21
Average no. eggs per female	154	—	126	26

adversely affected (table 7). It appears that relative humidities above 10% but below 50% have little effect on the flies. Between 50 and 60% there was some shortening of the life span and an apparent reduction in oviposition.

SUMMARY.—Cherry fruit fly larvae from six populations from central Washington bore 9 to 17 papillae on each thoracic spiracle. All therefore belong to the western subspecies, *Rhagoletis cingulata indifferens* Curran.

The pupae can withstand a temperature of 8° F. for 1 hour. Refrigeration of the pupae for 6 to 7 months resulted in 86% emergence while 15 to 17 months caused 65% mortality. During a 46-day developmental period, 88% of the pupae died at 70° F. while 92% died at 80° F.

Pupae held at 50° F. for 45 days failed to develop while 33% began to develop at 60° F. over the same period. At the same time, 86.5% were ready to emerge at 70° F. and 92% did emerge at 80° F. At 90° F., only 21.5% of the pupae produced flies and of those, 62% were deformed. The high temperature was detrimental to the pupae; 51.5% were killed and 25% did not develop. Some pupae were transferred from 80° F. to 90° F. and others from 90° F. to 80° F. at weekly intervals. The longer the periods at 90° F., the greater the pupal mortality.

Fruit infestations in an unsprayed orchard have varied from 1 to 85% in the past 8 years. Weather was the greatest factor but the size of the crop was of moderate importance. When early season temperatures were high, flies emerged rapidly and heavily infested the crops. Laboratory tests, using the actual air temperatures for one of the warmest and one of the coolest seasons, gave quantitative data on the development of ova, preoviposition period, and numbers of eggs oviposited. Results of field observations were thus confirmed. When adults were held at 90° F., relative humidities of 50 to 60% affected longevity and oviposition to some extent. Humidities above 75% were very adverse and greatly shortened longevity and oviposition.

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Effects of Soil Insecticides on Beans, Soybeans, and Cotton and Resulting Effect on Mite Nutrition[1]

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The field of soil insecticides is growing, and with its growth come problems with many facets and implications. Some general considerations that arise from their use are: 1) absorption and translocation in the plant, 2) persistence in the soil, 3) effect on specific plants or plant parts, 4) effect on soil microorganisms and 5) effect on composition of plants. It is entirely possible that any or all of the factors mentioned above may have some relationship or influence on the study presented here. Lilly (1956) reviewed soil insects and their control and refers to some of the problems encountered from the use of soil insecticides.

First, it is well to note the work reported in the general study of absorption and translocation occurring from the use of soil insecticides. According to Kozlova (1950), cabbage absorbed enough BHC from the soil in 24 hours to provide 46% control of the cabbage aphid. Starnes (1950) showed that soil treatments with parathion and BHC were absorbed and translocated to potato foliage. Terriere & Ingalsbe (1953) found that limited translocation of aldrin, dieldrin, chlordane, BHC, DDT, parathion and heptachlor occurred in potatoes. Gunther & Blinn (1956) include in their review the absorption of soil insecticides by root crops. Haines (1956) found evidence of lindane absorption and translocation in corn plants by bioassay of guttated liquid from the corn leaf.

The work of Allen *et al.* (1954) on the persistence of BHC, DDT and toxaphene in soil and the tolerances of

[1] This study was sponsored in part by a grant-in-aid from the Rockefeller Foundation and is the first in a series of papers dealing with the general subject of the effects of soil insecticides on plants and mite nutrition. Accepted for publication March 26, 1957.

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certain crops to their residues is also noteworthy. It was found that 4½ years after single applications approximately 38% of the DDT and 6% of the BHC remained in the soil, and there appeared to be a definite relationship between the amount of DDT in the soil and the stand, growth, and yield of cowpeas, oats and rye. Foster (1951) presented a study on the plant responses to DDT, BHC, chlordane, toxaphene and parathion. He found Abruzzi and Rosen rye and Stringless Black Valentine bean among those of the most sensitive observed.

Some of the effects of the insecticides on plants, whether beneficial or detrimental, are probably due to the influence of soil micro-organisms. Smith & Wenzel (1947) found that DDT at 200 pounds per acre did not injure microorganisms. BHC at 50 to 60 pounds proved to be fungicidal and it also decreased nitrifying bacteria. Chlordane was not so toxic to nitrifiers and fungi as BHC. Wilson (1954) obtained generally the same results. Simkover & Shenefelt (1951) found that at practical levels of BHC (1 pound per acre) and chlordane (10 pounds), BHC inhibited only the damping-off organisms but no differences occurred in the nitrogen-fixing bacteria. Bolen *et al.* (1954a, 1954b) found that BHC at 0.25 to 20 pounds per acre increased bacteria although streptomyces was decreased. Chlordane had no effect. Aldrin inhibited mold while dieldrin increased it. These workers considered the stimulation and inhibitions observed to be insufficiently intensive to materially influence fertility of the soil.

The wide usage of the chlorinated hydrocarbon insecticides on aerial parts of plants has generally created a mite problem. This has been attributed to the elimination of predators, the scattering of mite colonies, and perhaps an actual stimulation by the chemical *per se*. It follows that if the chlorinated hydrocarbons in the soil are absorbed and translocated to the aerial parts of the plant, the possible reaction to mite development should be considered. Besides the faint possibility of direct effect from the insecticide there is an even more important potential reaction: namely, the effect on the nutrition of the plant.

In work designed to study the effect of relatively low dosages of some of these materials on the growth and composition of corn, Rodriguez (1953, 1954) grew plants in artificial culture treated with aldrin and chlordane and found that phosphorus increased about 10% rather consistently with either material in the 1.0 p.p.m. range; sampling for analysis was done when the plants measured approximately 36 inches extended height. The same reaction occurred when soil was treated with aldrin at 4 pounds per acre in greenhouse-grown corn.

Klostermeyer & Rasmussen (1953) noted the effect of soil insecticide treatment on mite population and damage. Increased populations of *Tetranychus bimaculatus* on beans and potatoes were associated with increased dosages of DDT, BHC, lindane, aldrin and chlordane in the soil. Potatoes on field plots treated with 119 pounds or more of DDT per acre were killed by mites whereas potatoes in adjacent untreated plots were not damaged. They proposed that the effects were probably due to changes in plant nutrition and composition induced by the insecticides in the soil. The fact that mites are variously affected by the nutrition of the host plant has been demonstrated (Rodriguez 1951, Rodriguez & Rodriguez 1952).

The purpose of this study was to investigate effects of some of the materials used as soil insecticides on the composition of such plants as beans, soybeans and cotton, and to correlate mite development with the nutritional changes of the host plant.

EXPERIMENTAL.—*Greenhouse studies.*—All of the work was done in the greenhouse. The host plants Black Valentine beans, Clark soybeans, and Delta Pine cotton, were planted in finely screened top soil (Maury silt loam), using 6 to 8 inch clay pots. Treatments were calculated on the basis that 6 inches of the average soil over the surface area of 1 acre equals about 2 million pounds. The general plan was to grow the different host plants in a wide range of insecticide rates at various times during the year to determine the effect of the materials on the host plants and the resulting effect on plant-feeding mites. Usually each experiment contained nine treatments and four replicates (pots) per treatment. During the course of 2 years, some 38 experiments were conducted. The insecticides which were added as wettable powders and thoroughly mixed in each pot before planting, included DDT, BHC, lindane, aldrin, dieldrin and chlordane. Table 1 gives the general ranges of each insecticide used in a series of tests made on each type of plant. A set number of seeds were planted for each type of plant for the entire series, and dry weight of the foliage was recorded after leaflets were detached for mite introductions. Plants were never allowed to reach the bloom stage.

Mite technique.—Leaves, chosen from the first trifoliate in Black Valentine beans and soybeans, and from leaves of a comparable rank in cotton, were detached from the plants and taken into the laboratory where the detached leaf culture method was used in culturing the mites. The technique as set forth (Rodriguez 1953a) was modified in that the leaf disks rested on water-saturated filter paper instead of being floated on sucrose solution. Two leaflets (replicates) per pot were detached and five young female adult mites were introduced to each disk. The petri dish was not closed totally, as some air circulation was needed to prevent condensation on top of the petri dish. The humidity inside the dish was estimated at saturation but the relative humidity of the laboratory was not controlled and it averaged 70% in the summer and about 35% during the winter. The temperature was 82° F. ± 2 for the experimental periods during the summer and a rather consistent 80° F. was maintained during the winter months. The developmental period for both species tested, *Tetranychus telarius* (L.) and *T. tumidus* Banks, was 7 to 8 days. The resulting progeny

Table 1.—Ranges in amounts of insecticides applied in the tests.[a]

INSECTICIDE	HOST PLANT		
	Beans	Soybeans	Cotton
DDT	5–2000	5–2000	5–2000
Aldrin	5–1000	10–1000	10–1000
Dieldrin	10–1280	10–1280	5–320
Chlordane	40–640	40–640	—
BHC	2.5–320	2.5–320	5–240
Lindane	20–320	20–320	—

[a] Expressed in pounds of active ingredient per acre.

were refrigerated upon the termination of each series and counting of mites followed. *T. tumidus* was tested on cotton only.

ANALYSES.—*Foliage.*[3]—Air-dry foliage ground in the Wiley micro-mill was analyzed for nitrogen, phosphorus and potassium using standard methods (Anon. 1940). At the beginning, foliage samples from each replicate were analyzed separately. An evaluation of early results indicated that this procedure was not necessary, and thereafter two replicates were combined so that 18 combined samples were analyzed for each experiment.

Statistical.—Analysis of variance was performed on the results of the mite counts, using multiple classification on the two mite development counts from each experiment. For the calculation of correlation coefficients, mite counts, combined if possible, otherwise individual, were correlated with the corresponding nitrogen, phosphorus or potassium contents of the foliage samples.

RESULTS AND DISCUSSION.—*Studies with soybeans.*—The dosages of DDT on soybeans ranged from 5 to 2000 pounds per acre of the active ingredient. Results from tests in the relatively lower ranges are typified in figure 1 (5 to 640 pounds per acre). Mite development was depressed slightly at the 5 to 20 pound range as nitrogen in the leaves was maintained relatively steady but mites returned to their original level at 40 pounds and decreased little thereafter. Nitrogen, depressed at 20 to 80 pounds, was increased beyond the original level at about 200 pounds. Phosphorus increased slightly at the lower levels and then levelled off. Plant growth was stimulated at 10 pounds per acre and depressed noticeably at 2000 pounds (e.g., one experiment weighed 0.40 gm. per plant dry weight of foliage on the check plot, 0.44 gm. and 0.20 gm. on the 10- and 2000-pound levels, respectively). Plant growth evidently was not affected greatly at the 1000-pound level. Mite development at the higher dosages did not change appreciably from the dosages under 1000 pounds.

The BHC and lindane studies are typified by those shown in figure 2. The general reactions were similar; mite development and potassium decreased drastically as nitrogen and phosphorus increased. The dip in the nitrogen curve with BHC at 10 to 20 pounds was matched in the lindane treatment at 20 pounds, but nitrogen increased as the dosage of insecticide increased; e.g., at 320 pounds, it was 184% over the untreated check. Mite population was significantly correlated negatively with nitrogen and phosphorus and positively with potassium in the BHC study. Although the same general effect on the elements was noted in the lindane test, the differences were less in degree; the mite population differences were significant, but the correlations of mites with elements were not significant. At relatively low dosages of BHC, some stimulation of plant growth was evident; at 40 pounds and above, deleterious effects were observed, as they were at the 160- and 320-pound dosages of lindane (table 2). The fact that BHC has more profound effects on plant growth and development than some other

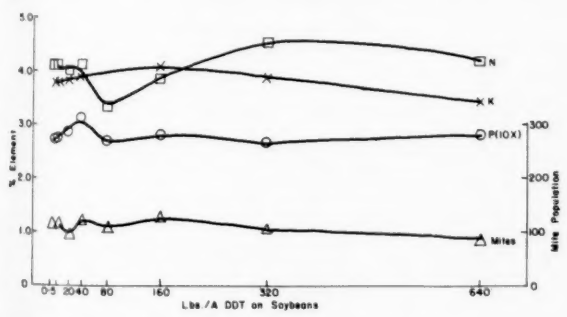

FIG. 1.—Effect of DDT on soybeans as typified by this experiment showing N, P, and K absorption (% element). Phosphorus content is represented as 10 times the actual amount. *Tetranychus telarius* population is expressed as the average number of progeny resulting from five females during a given developmental period. F value for mites = 1.55.

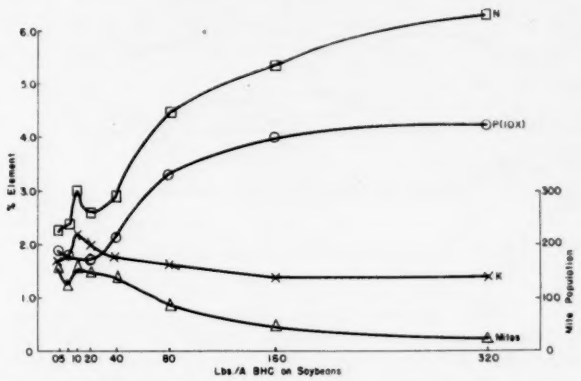

FIG. 2.—Effect of BHC on soybeans as typified by this experiment showing N, P, and K absorption (% element). Phosphorus content is represented as 10 times the actual amount. *Tetranychus telarius* population is expressed as the average number of progeny resulting from five females during a given developmental period. F value for mites = 6.70**; r value N with mites = −.950**; r value P with mites = −.976**; r value K with mites = +.678**.

Table 2.—Dry weight of foliage[a] of plants grown in soil treated with several insecticides at different dosage levels.

POUNDS PER ACRE	INSECTICIDE				
	BHC	Lindane	Aldrin	Dieldrin	Chlordane
	Black Valentine Beans				
0	0.35	0.23	0.23	0.25	0.29
10	0.36	—	—	0.23	—
20	0.36	0.20	—	0.24	—
40	0.23	—	0.19	0.23	0.29
80	0.15	0.22	—	0.24	0.25
160	0.09	0.19	0.24	0.23	0.26
320	0.05	0.17	0.21	0.21	0.26
640	—	—	0.24	0.20	0.24
	Soybeans (Clark)				
0	0.28	0.32	0.33	0.33	0.36
10	0.26	—	—	0.37	—
20	0.32	0.26	—	0.39	—
40	0.17	—	0.31	0.34	0.31
80	0.11	0.32	—	0.36	0.33
160	0.10	0.24	0.26	0.34	0.31
320	0.06	0.12	0.27	0.30	0.27
640	—	—	0.29	0.25	0.28

[a] Expressed in average number of grams per plant.

[3] The assistance of Mr. Jack R. Todd, Department of Agronomy, in the foliage analysis is gratefully acknowledged.

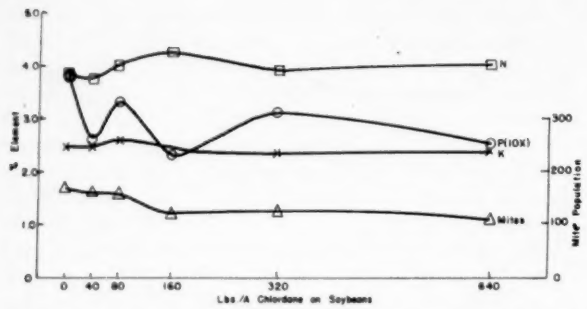

FIG. 3.—Effect of chlordane on soybeans as typified by this experiment showing N, P, and K absorption (% element). Phosphorus content is represented as 10 times the actual amount. *Tetranychus telarius* population is expressed as the average number of progeny resulting from five females during a given developmental period. F value for mites=3.43**.

chlorinated hydrocarbon insecticides has been established by other workers (Ball 1956, Chulski 1948, Scholes 1953).

The effects of aldrin and dieldrin on the elements were much the same. Nitrogen was increased slightly at the lower to mid-levels; this increase continued in the dieldrin studies at levels above 320 pounds, nitrogen being as high as 37% over the check plot at 1280 pounds per acre. Phosphorus absorption was similar to that of nitrogen. Both aldrin and dieldrin effected a slight depression in mites which was not significant. Figure 3 (chlordane) shows the increase in nitrogen typical of this group, but chlordane decreased phosphorus at all levels and depressed populations significantly at the middle to higher levels (160 to 640 pounds). Stimulation of plant growth was observed at the lower levels of these insecticides in most of the tests and dieldrin increased the actual dry weight of the foliage by 18% over the untreated check at the 20-pounds-per-acre level (table 2). This effect has been demonstrated in other host plants; for instance, Stone & Smith (1951), growing corn in the field, noted that chlordane, aldrin and dieldrin stimulated the growth of plants nearly a foot for the first month of their growth

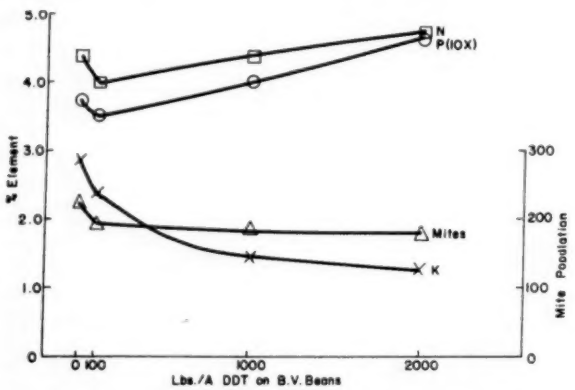

FIG. 4.—Effect of DDT on Black Valentine beans as typified by this experiment showing N, P, and K absorption (% element). Phosphorus content is represented as 10 times the actual amount. *Tetranychus telarius* population is expressed as the average number of progeny resulting from five females during a given developmental period. F value for mites=0.89; r value K with mites=+.757*.

period. Rodriguez (1953b, 1954) found that aldrin and chlordane, in additions to corn grown both in artificial culture and in soil in the greenhouse, stimulated plants rather consistently at the rates of 1 to 3 p.p.m. or 1 to 4 pounds per acre. This effect was demonstrated in both height and weight of the plants.

Black Valentine beans.—The effect of DDT on Black Valentine beans was distinctive in that DDT affected nitrogen and phosphorus similarly, both being depressed at the 5- to 100-pound level, then increased within the 100- to 200-pounds-per-acre range. Potassium was decreased with this increase of nitrogen and phosphorus at the upper levels. Scrutiny of several tests showed mite development somewhat erratic, increasing above the checks in some cases and in others decreasing as shown in figure 4. This phenomenon could not be explained at this time; however, potassium content was found to be positively correlated with mite development where the latter was depressed (fig. 4). Little or no stimulatory effect was noted on the plants at the very low dosages; at 1000 pounds per acre dry weight of foliage was decreased nearly 50%. Black Valentine beans have generally been found to be relatively sensitive to DDT (Foster 1951, Thurston 1953).

BHC again demonstrated the most profound effect on the elements of any of the materials tested (fig. 5). Mite development was increased over the untreated check at levels as high as 160 pounds per acre. Comparison of the action of BHC on soybeans and Black Valentine beans showed the effects on the elements to be similar, whereas the effect on mite development differed, in that in soybeans no populations were higher than the check. In the lindane studies, nitrogen and phosphorus fell to a low level at the 160-pound rate and increased again at the 320-pound rate. Potassium increased slightly at 20 pounds per acre, then decreased continuously. Mite development increased sharply at the 20-pound level and remained high through the 160-pound level before decreasing. The population demonstrated a definite, though not significant, negative relationship with phosphorus and a positive relationship with potassium.

Aldrin and dieldrin effected a slight dip in the nitrogen

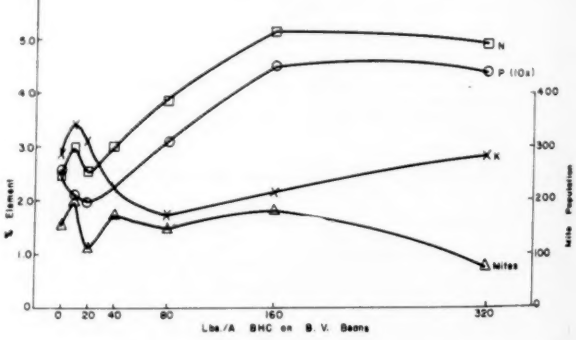

FIG. 5.—Effect of BHC on Black Valentine beans as typified by this experiment showing N, P, and K absorption (% element). Phosphorus content is represented as 10 times the actual amount. *Tetranychus telarius* population is expressed as the average number of progeny resulting from five females during a given developmental period. F value for mites=1.01.

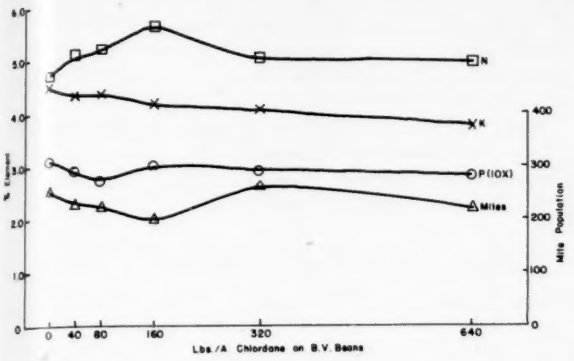

FIG. 6.—Effect of chlordane on Black Valentine beans as typified by this experiment showing N, P, and K absorption (% element). Phosphorus content is represented as 10 times the actual amount. *Tetranychus telarius* population is expressed as the average number or progeny resulting from five females during a given developmental period. F value for mites = 1.06.

and phosphorus curves. Mites showed a significant increase over the untreated check. Potassium absorption was not consistent. Data on dry weight of foliage (table 2) do not indicate stimulation of the plant from the addition of either material; photographic records, however, show an apparent stimulation of plant height by aldrin and dieldrin treatments as compared with the untreated check.

The reaction of chlordane was typical in that mite development decreased as absorbed nitrogen increased. This was true up to the 160-pound level, after which the two curves reversed their directions (fig. 6). At 320 pounds only, chlordane treatment resulted in slightly more mites than the untreated check. Potassium was depressed gently but steadily by added chlordane. As in aldrin and dieldrin, data on dry weight of foliage do not indicate stimulation of growth.

Studies with cotton.—Treatments of DDT on cotton ranged from 10 to 2000 pounds per acre and had no appreciable effect on the growth of the cotton plant; the 1000-pound level produced plants apparently the same size and weight as those treated at 10 pounds. Phosphorus, the only element affected to any marked extent, was depressed by DDT at all levels (fig. 7). *T. tumidus*[4] increased slightly at the 1000-pound level, otherwise was virtually unaffected. The effect of DDT-treated cotton on *T. telarius* was not tested.

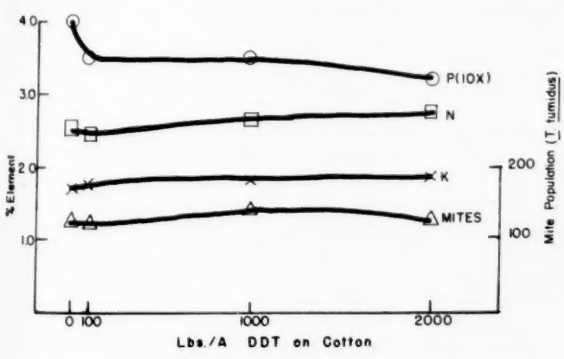

FIG. 7.—Effect of DDT on cotton as typified by this experiment showing N, P, and K absorption (% element). Phosphorus content is represented as 10 times the actual amount. *Tetranychus tumidus* population is expressed as the average number of progeny resulting from five females during a given developmental period. F value for mites = .40.

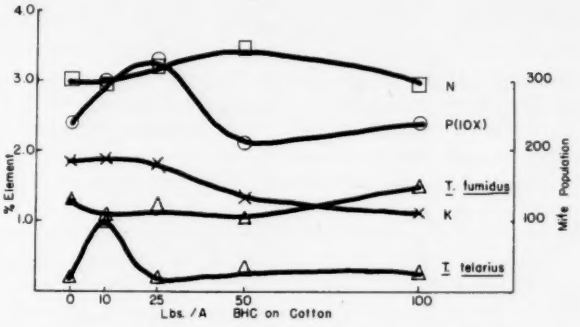

FIG. 8.—Effect of BHC on cotton as typified by this experiment showing N, P, and K absorption (% element). Phosphorus content is represented as 10 times the actual amount. Mite population is expressed as the average number of progeny resulting from five females during a given developmental period. F value for *T. telarius* = 9.74**; F value for *T. tumidus* = .58.

BHC again exerted a relatively marked but not always consistent effect on absorption of elements. Nitrogen was apparently decreased by 5 to 20 pounds per acre but this tendency was reversed at levels up to 70 pounds. In one test, absorbed phosphorus was positively correlated with the development of *T. telarius;* in another (fig. 8) it was not. Potassium generally was depressed with increased BHC dosages. Comparison of the development of the two species *T. tumidus* and *T. telarius* (fig. 8) demonstrates that their reactions to BHC and/or plant composition differed. *T. telarius* was stimulated at the lower levels of treatment, as on Black Valentine beans, while the only increase noted in the population of *T. tumidus* was a slight one at the 100-pound level.

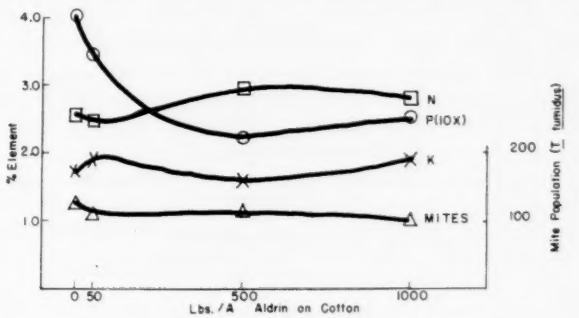

FIG. 9.—Effect of aldrin on cotton as typified by this experiment showing N, P, and K absorption (% element). Phosphorus content is represented as 10 times the actual amount. *Tetranychus tumidus* population is expressed as the average number of progeny resulting from five females during a given developmental period. F value for mites = 1.11; *r* value P with mites = +.517.

As in the Black Valentine beans, aldrin effected a slight dip in nitrogen absorption at the 50-pound level (fig. 9).

[4] This species was supplied through the courtesy of H. Bruce Boudreaux, Louisiana State University.

Phosphorus was decreased sharply up to 500 pounds. Potassium increased slightly at the 50- and 1000-pound levels. Development of *T. tumidus* was related positively to absorbed phosphorus, being depresesd at treatments of 50 pounds and higher. An apparent stimulation of plant growth was observed at the 10-pound rate and a slight depression was seen at the 500-pound rate.

Treatments with lindane and chlordane were omitted in the cotton studies because of the general similarity of lindane to BHC and of chlordane to aldrin, and dieldrin.

SUMMARY.—The effects of DDT, BHC, lindane, aldrin, dieldrin and chlordane as soil insecticides on the composition of Black Valentine beans, Clark soybeans and Delta Pine cotton were investigated. Plants were grown in pots in the greenhouse and the nitrogen, phosphorus and potassium contents were correlated with mite populations. *Tetranychus telarius* (L.) and *T. tumidus* Banks were used, the latter on cotton only. Mite development was measured by counting the progeny developing from five adult female mites which had been introduced to detached leaf disks.

On soybeans, DDT depressed mites slightly at 5 to 20 pounds per acre, and depressed nitrogen at the 80- to 160-pound levels. Plant growth was stimulated at 10 pounds per acre. BHC exerted profound effects, decreasing mite populations and potassium sharply at high levels as nitrogen and phosphorus increased; significant correlations of mites with elements were found, negative with nitrogen and phosphorus, positive with potassium. Lindane effects were similar but less significant. Low dosages of BHC stimulated plant growth and high dosages depressed it; only the latter effect was noted with lindane.

Effects of aldrin, dieldrin and chlordane on the elements were similar, being generalized as a slight nitrogen increase at lower to middle levels (20 to 160 pounds per acre), with a slight depression in mite development, significant only with chlordane. Plant growth stimulation was noted in most tests at the lower levels.

On Black Valentine beans, DDT decreased nitrogen and phosphorus at the 5- to 100-pound range, then increased these elements while decreasing mites at the higher levels. Potassium, also decreased, was generally positively correlated with mite development. Little or no stimulation of plant growth at low dosages occurred, and phytotoxicity was noted at moderate dosages.

In Black Valentine beans, as in soybeans, BHC affected plant composition strikingly and lindane less so; however, mite development was increased by BHC at levels as high as 160 pounds per acre. In the lindane test, mite development was correlated negatively with nitrogen and phosphorus, positively with potassium.

Unlike in soybeans, aldrin and dieldrin depressed nitrogen and phosphorus slightly in Black Valentine beans, and increased mites over the untreated check. Plant stems appeared elongated at the lower levels. Chlordane affected a typical mite-nitrogen reaction in that nitrogen increased as mites decreased up to 160 pounds per acre and the curves then reversed directions.

In cotton, DDT had little effect on plant growth, *T. tumidus* development, or absorption of elements except phosphorus, which was depressed by all levels of DDT. BHC again influenced plant composition to a greater degree than the other insecticides, though erratically in the case of phosphorus. Nitrogen was decreased by low levels as plant growth was stimulated, and at higher levels the reverse was true. *T. telarius* and *T. tumidus* varied in reactions to BHC, only the former showing increased development at low dosages.

Aldrin in cotton depressed nitrogen slightly and phosphorus considerably. *T. tumidus* development, also depressed, was positively correlated with absorbed phosphorus. Effects on plant growth were slight.

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Light Oil with Parathion or Malathion for Control of Black Scale on Oranges[1]

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Black scale, *Saissetia oleae* (Bern.), has decreased in importance as a citrus pest since the parasite *Aphycus helvolus* (Compere) was introduced into California in 1937 (Flanders 1942), but infestations still become serious at times. A general outbreak followed the cold winter of 1949–50, probably because of the susceptibility of the parasite to low temperatures. In years of normal weather *helvolus* is less effective in the interior sections, where there is a single brood, than in coastal sections, where there is some overlapping of generations to provide a succession of stages suitable for parasitization. In 1954 and 1955 there were heavy scale infestations in parts of several counties where the insect is single-brooded.

Sprays of about 1.75% of light medium oil following the major hatch in the summer have generally given satisfactory control of black scale. They are also effective against the California red scale, *Aonidiella aurantii* (Mask.), and the citrus red mite, *Metatetranychus citri* (McG.). However, there are disadvantages to the use of oil sprays on navel orange trees. Oils may remove the wax bloom and sometimes retard coloration of the fruit. More important is their tendency to increase water spot, which sometimes follows wet weather in the winter. Ebeling *et al.* (1938) found that navel oranges in plots in two groves sprayed with 1.67% light medium oil showed 18 and 37% water spot and the untreated plots only 5 and 11%.

DDT with kerosene or DDT emulsions have been very effective against black scale and have not harmed the trees or fruit. They do not control California red scale and may aggravate citrus red mite and aphid problems; therefore their use has been limited.

Parathion and malathion do not accentuate water spot and are effective against red scale, but are weaker than light medium oil or DDT on black scale, and at economical dosages are toxic only to the very young stages. For this reason an even hatch and close timing of applications are necessary for good control in the single-brooded areas. No timing has given satisfactory results in the double-brooded areas or in the single-brooded areas when the hatching period was prolonged.

There is need for a material that is effective against both black and red scales, and is not injurious to navel orange trees and fruit. Earlier work suggested that a special light oil with additives might fill this need. Such an oil with 0.12 or 0.25 pound of parathion was found to be just as effective against red scale as an equal amount of light medium or heavier oil with parathion (Cressman *et al.* 1950, 1954), interfered little with coloration of fruit, and did not reduce soluble solids of oranges (Cressman 1955). When 1.75% of oil plus parathion was used, the light-oil mixture caused about as much leaf and fruit drop as light medium or medium oils, but when the oil concentration was reduced to 1%, the light oil plus parathion was less hazardous than the heavier oils. Studies on the use of this special light oil with parathion or malathion for black scale in California beginning in 1953 are reported in this paper.

MATERIALS AND METHODS.—The special light oil had the following characteristics:

Viscosity at 100° F.	50–55 seconds Saybolt
Unsulfonatable residue	90% minimum
Distillation at 636° F.	82% minimum

It was made emulsifiable with an emulsifier mixture that is present in one of the commercial spray oils. Parathion and malathion, 25%, wettable powders were used with the special light oil, but malathion emulsions were combined with the light medium oil in 1955. Concentrations are stated in terms of the active ingredients.

Mortality counts on black scales were made 3 to 4 weeks after treatment, and the infestation was checked the following summer. Since the final infestation was always very light, it was measured by counting the living scales found in 2 hours' inspection of 10 trees per plot. About 2000 twigs could be examined in this time. Both California red scale and citrus red mite infestations were also measured when inspection indicated significant populations, the first by methods described by Cressman & Broadbent (1944), the second by running leaves through a brushing machine described by Henderson & McBurnie (1943) and counting the mites and eggs collected on adhesive-coated plates.[2]

The amount of water spot on navel fruit was estimated by examining fruit on the north half of trees, with samples limited to oranges that could be reached from the ground. Water spot is generally worse on the north exposure and top of the trees than on the south and lower parts. The counts therefore are a measure of the relative amount of injury in different plots, but not of the total fruit affected.

1953 EXPERIMENTS.—Preliminary experiments were made in a Valencia orange grove near San Fernando.

[1] Accepted for publication March 26, 1957.

[2] Red mite counts were made by Francis Munger.

Black scale here is single-brooded. No treatment had been made in 1952, and by the following winter it was evident that both black and red scales were sufficiently numerous to cause severe damage before harvest unless an emergency treatment was applied. On March 18, 1953, two plots of about 60 trees each were sprayed by oscillating boom with 1.0 gallon of oil plus 0.12 pound of parathion per 100 gallons, special light oil in one plot and light medium in the other. At the same time the owner sprayed the rest of the grove with a mixture of 2 gallons of kerosene, 0.5 pound of DDT, and 0.2 pound of parathion per 100 gallons. Many black scales were in the rubber stage when treated.

No mortality counts were made, but in 2 hours' inspection in the three treatments the following summer only three live black scales were found. The red scale infestation averaged one scale per fruit before spraying and less than 0.2 at harvest in August, with no significant difference between the three treatments. A heavy infestation of aphids occurred in the spring, and treatment was necessary by June 1. The aphids were much worse in the part of the grove sprayed with kerosene, DDT, and parathion than in the experimental plots. No red mite infestation developed. There were no adverse effects of the sprays on trees or fruit.

On August 21, 1953, other sprays were applied by hand to a heavily infested Valencia orange grove in the double-brooded area near Capistrano. Three plots of 16 trees each were sprayed with 1 gallon of special light oil plus 0.12 pound of parathion, one plot with the same oil plus 0.25 pound of parathion, and three plots with 1.75 gallons of light medium oil alone. Black scale mortality counts included 1000 scales on twigs and 2000 on leaves in each plot. Final population counts were made in July 1954. A light red mite infestation was present in some plots by December, and mite samples were taken on December 3, after which all plots were sprayed with ovex.

The results, given in table 1, show that all treatments gave good control of the black scale. The final infestations were too low to show any significant differences between treatments, and indicated that concentrations of parathion much above 0.12 pound per 100 gallons might not be needed with this amount of oil. One per cent of light oil with parathion was less effective against citrus red mites than 1.75% of light medium oil alone. A light infestation of red scales was satisfactorily controlled by all treatments.

1955 EXPERIMENTS.—Other sprays were applied by oscillating booms in two groves near Corona that were very heavily infested with single-brooded black scales. Grove A, navel oranges, was sprayed on August 22, 1955. Each mixture shown in table 2 was applied to two plots of three rows of 13 trees. The arrangement of plots was such that 12 adjacent rows received 1% of light oil with additives. Insect counts were taken in the middle row of each plot. Plots in grove B, a mixed planting of Valencia and navel oranges, were sprayed on August 25. Each mixture was applied to two plots consisting of two rows of 40 trees. Insect and mite infestations were sampled on the inner halves of the pair of rows making up each plot in order to minimize the effect of drift of the spray applied to the adjacent rows.

The California red scale infestation was light in both groves before treatment. Inspection the following summer showed no increase. No red mite infestation developed in any plots in grove A. Results on black scale in both groves and on the citrus red mite infestation in grove B are shown in table 2.

The initial mortalities of black scales were very high in all plots, and the final infestations were too low to show any difference between treatments. All sprays again gave very good control in both groves. The slightly better result in grove A was probably due to a heavier spray application made there. In grove B the mite infestation was heavier in the plots treated with light oil. The difference between the plots treated with parathion and malathion with light oil was not significant.

In grove B leaf drop was moderate to heavy in the plots sprayed with light medium oil plus malathion, and very light in the special light oil plots. In grove A, 2,4-D was added to the light medium oil, and there was little leaf drop anywhere. In both groves fruit in the special light oil plots lost little or none of the wax bloom, but where light medium oil was used the oranges retained very little bloom and had an oily feel as late as January.

The outstanding difference between treatments was in the incidence of water spot. Following heavy rains in January water spot became rather general in oil-sprayed navels. In grove A, 12.5% of the fruit sprayed with 1.8% light medium oil was affected, as compared with only 0.4% of the fruit sprayed with 1% of special light oil. In grove B fruit was picked soon after the rain and no data on water spot were obtained.

DISCUSSION.—Earlier work had shown that 1 gallon of special light oil plus 0.12 pound of parathion per 100 gallons was at least equal to the conventional oil sprays

Table 1.—Effect of sprays applied on August 21, 1953, on double-brooded black scale and citrus red mite populations.

Materials per 100 Gallons	Black Scales: Per Cent Mortality Sept. 1953	Black Scales: Scales per 2-Hour Inspection July 1954	Citrus Red Mites per Leaf Dec. 1953: Eggs	Citrus Red Mites per Leaf Dec. 1953: Mites
Light medium oil 1.75 gallon	99.5	14	0.1	0.02
Light oil 1 gallon plus parathion—				
0.12 pound	99.3	28	8.0	1.5
0.25 pound	99.7	1	3.7	1.0

Table 2.—Effect of sprays applied in August 1955 on single-brooded black scale and citrus red mite populations.

Materials per 100 Gallons	Black Scales: Per Cent Mortality Sept. 1955 Grove A	Black Scales: Per Cent Mortality Sept. 1955 Grove B	Black Scales: Scales per 2-Hour Inspection May 1956[a] Grove B	Citrus Red Mites per Leaf, Grove B May 1956: Eggs	Citrus Red Mites per Leaf, Grove B May 1956: Mites
Light medium oil 1.8 gallon +malathion 0.25 pound	100.0	98.0	19	0	0.01
Light oil 1 gallon plus—					
Parathion 0.15 pound	99.9	99.8	30	1.8	.3
Malathion 0.25 pound	99.9	99.7	45	4.6	1.8

[a] Only three scales found in 2 hours' examination in grove A.

containing 1.7–1.8% of light medium or medium oil alone on California red scale, but weaker on citrus red mite (Cressman *et al.* 1954). In the black scale experiments also the light-oil combinations controlled light infestations of red scale but were inferior to the light medium oil on red mite. Since Valencia oranges are more tolerant than navels to oil sprays, the full dosage of light medium oil would probably be preferred on Valencias in order to control red mites as well as black and red scales. On navel oranges parathion without oil would be the best treatment for red scale alone. When navels require treatment for black scale and red scale is light to moderate, the mixture of special light oil with 0.12–.15 pound of parathion or 0.25 pound of malathion per 100 gallons appears promising as an economical method of controlling both pests without increasing susceptibility to water rot. Further testing of these combinations on a semicommercial scale is under way.

SUMMARY.—Sprays of 1% of a special light oil plus parathion or malathion were compared with 1.8% light medium oil alone and with malathion for control of black scale, *Saissetia oleae* (Bern.), on oranges in California.

One per cent of light oil with 0.12 or 0.15 pound of parathion or 0.25 pound of malathion was as good as the 1.8% of light medium alone or with malathion on black scale, but less effective on the citrus red mite, *Metatetranychus citri* (McG.). Control of light infestations of California red scale, *Aonidiella aurantii* (Mask.) was satisfactory.

The light oil combination had little effect on the appearance of the fruit and did not accentuate water rot in navel oranges.

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The Biology and Control of the Maggot Complex Associated with Spinach[1]

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The presence of insect fragments in canned fruits and vegetables has been an important problem to the processing industry for many years. In the processing of spinach, the primary problem has been associated with the presence of the larvae of the spinach leaf miner, *Pegomya hyoscyami* (Panz.), in the stems and leaves at the time of harvest. An outstanding example of the "political and economic" importance of this problem was published by Austin (1952). He reported that the 1950 spinach pack in Arkansas was reduced from an estimated 3,000,000 to 100,000 cases by the presence of "stem worms."

Isely (1946) reported that the green peach aphid, *Myzus persicae* (Sulz.), when present on spinach can be reduced below the point where feeding injury is apparent but still will be a serious problem in the canned pack. The relationship of fertilizers to seed-corn maggot (*Hylemya cilicrura* (Rond.)) injury to spinach seedlings was reported by Reid (1936).

In 1933, Smith first discussed the feeding activities of the spinach "bud worm" which was later identified as the seed-corn maggot, *Hylemya cilicrura* (Rond.). Correspondence with other investigators indicates that the same problem exists in New York, Oklahoma and Texas. The purpose of this study was to investigate the species composition and biology of the insects infesting the buds of spinach and to evaluate various control practices.

The typical type of injury resulting from the maggot infestations is shown in figure 1. The feeding of the larvae causes distortion of the leaves which may develop necrotic lesions that hasten the deterioration of the plant. When the infestation develops early in the growth of the crop, the plant may be completely destroyed by secondary rot organism invaders. In the processing industry, the most troublesome problem is the presence of the larvae in the young seed heads. The larvae are pushed up with the seed head as it develops and are contained in the spinach harvested with commercial harvesting machinery.

Biology and control studies were carried on during 1954, 1955 and 1956 in cooperation with the Gerber Products Co., Fremont, Mich. All of the work was conducted in the Grant area of Michigan where spinach is grown exclusively on organic soils. The crop is usually seeded during the third week of April and harvested the second week of June. The Giant Thick-Leaf variety was

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FIG. 1.—Spinach plant showing typical maggot injury to the bud and expanding leaves.

grown during the 3 years of the investigation. Overhead sprinkler irrigation was used to maintain adequate soil moisture. The crop was not cultivated. Yields averaged 12 tons per acre during the 3 years of the study.

BIOLOGY AND SPECIES COMPOSITION.—Samples of infested spinach were brought into the laboratory and placed in rearing chambers to determine the species composition of the larval population. Emerged adults were removed from the chambers daily. Table 1 presents the results of the 3-year study of species composition. No adults emerged from maggot infested spinach caged in 1956.

During 1954 and 1955, a single species of *Drosophila*, *Scaptomyza graminum* Fallen, was the major species present in the maggot complex. The seed-corn maggot, *Hylemya cilicrura* (Rond.), the false stable fly, *Muscina stabulans* (Fall.), and *Muscina assimilis* (Fall.) were important contributors to the infestation. The remaining species were secondary pests which were probably attracted to the decaying areas of the plants. Two families with parasitic representatives were found in the reared specimen populations. Field observations indicated that the 1956 insect complex was comparable in species composition with those of 1954 and 1955.

There was no correlation between the maturity of the crop and the build-up of a maggot population. Generally the later plantings were more heavily infested than the early seedings. This was especially true in 1956 when no

Table 1.—Insects reared from infested spinach.

Taxonomic Category	Number of Fields Represented	Total Specimens Collected		Per Cent of Specimens Collected
		1954	1955	
DROSOPHILIDAE				
Scaptomyza graminum Fallen	4	37	95	71
MUSCIDAE				
Hylemya cilicrura (Rond.)	5	12	8	10
Hylemya brassicae (Bouche)			1	
Muscina stabulans (Fall.)	3	4	10	7
Muscina assimilis (Fall.)	2	19		10
CHLOROPIDAE				
Elachiptera costata (Lw.)	1	1		—[a]
Oscinella melancholica Beck	1		1	—[a]
Hippelates bishoppi Sabr.	1		1	—[a]
SCATOPSIDAE				
Scatopse fuscipes Mg.	1	1		—[a]
SPHAEROCERIDAE				
Leptocera longicasta Spuler			2	1
LYCORIIDAE				
Bradysia sp.	2		2	1
EULOPHIDAE				
Hyssopus rhyacioniae (Gah.)	1	1		—[a]
BRACONIDAE				
Aphaereta auripes (Prov.)		1		

[a] Less than 1% of total.

maggots were found in the first plantings harvested. The later plantings in 1956, however, were heavily infested.

Extensive populations of *Drosophila* and seed-corn maggot flies appeared within a 12- to 18-hour period in certain fields. It was observed that these flies developed on the lower leaves of the mature crop just before harvest. Often several of these leaves rot and provide an ideal substrate for larval development. The adult populations continued to build up in fields which were sprayed at 3-day intervals with parathion.

During the 3 years of the investigation, there was no evidence as to what species of fly appeared first in the field. Generally the mixed populations built up with equal rapidity. Seventy to 80% of the eggs were laid on the soil in close proximity to the plant. The remaining eggs were deposited on the leaf petioles, and in a few instances, directly on the growing tip. Apparently the young larvae migrated to the expanding buds immediately after hatching.

CONTROL RESULTS.—Control studies were begun in 1954 and continued during 1955 and 1956. The 1954 plots consisted of six, 50-foot rows replicated twice. In 1955 and 1956, each plot was composed of nine 70-foot rows replicated four times. Data were taken by pulling and examining individual plants chosen at random from the three inner rows of each plot. In 1954, four applications were made at 7-day intervals beginning May 13 except as noted in table 2. In 1955, foliage applications were made on May 6, 11, 19 and 30. In addition to the foliage treatments, separate plots were established on which broadcast soil treatments were applied and worked into the upper 4 inches of the soil on April 15 previous to seeding the spinach. Applications were made on June 19 and 21 to the 1956 plots. In 1956, a duplicate experiment was established on early plantings but an infestation did not develop. Four applications were applied to the early planting and residue samples were harvested.

All sprays were applied with a tractor-mounted low-

Table 2.—Effectiveness of several organic insecticides applied to the foliage as wettable powders for control of the maggot complex in spinach. 1954.

Material and Per Cent Active Ingredient	Pounds of Toxicant per Acre	Per Cent of Plants Infested		Per Cent Control	
		June 15	June 21	June 15	June 21
DDT, 75	1.0	14	6	74	83
Lindane, 25	.25	12	18	77	51
Malathion, 25	.75	14	14	74	60
Parathion, 15 (4 sprays)	.15	42	44	22	20
Parathion, 15 (2nd, 3rd, and 4th spray)	.15	24	18	55	51
Parathion, 15 (3rd and 4th spray)	.15	40	29	26	9
Untreated		54	36		

pressure sprayer. The rate of application was 50 gallons of spray per acre applied at 40 pounds pressure. A single nozzle was located above each row so that all of the spray cone was directed downward into the whorl of the plant. In 1955, the granulated materials were applied by hand directly over the row. A Gandy High-Low granule applicator was used to apply the granulated materials during 1956. All of the feed holes with exception of those directly over the row were closed with masking tape.

The results of the 1954 control studies are outlined in table 2. In 1954, some of the plots were heavily infested while adjacent plots contained only a few larvae. As a result of this heterogeneous infestation, it was impossible to draw definite conclusions from the data. It was apparent that all of the materials reduced the larval population but not in sufficient magnitude to be commercially practical.

Table 3 presents the results of the 1955 soil treatments. Both dieldrin and heptachlor significantly reduced the maggot population. Dieldrin was the most effective material, but only resulted in a 55% reduction.

Table 3 also presents the results of the foliage treatments made during 1955. All of the materials with the exception of the granulated malathion were applied as sprays. Each treatment, except the Phosdrin and the malathion spray, significantly reduced the larval population. The granulated malathion treatment provided the most satisfactory means of control during 1955 and was the first indication that practical control might be possible. Since the dieldrin spray was only slightly less effective than the granulated malathion it was postulated that a granulated dieldrin treatment might provide satisfactory control.

In 1956 granulated dieldrin, heptachlor, parathion and malathion were included in the study. The results of the 1956 study are also presented in table 3. The granulated materials were the most effective treatments in the 1956 tests. Granulated dieldrin provided 99% control and granulated heptachlor was only slightly less effective giving 90% reduction. Generally the chlorinated hydrocarbon materials were more effective than the organic phosphate insecticides. Dow ET-57 (sampled as Dow ET-14), Thiodan and Dipterex all failed to give significant control. The entire plot layout was sprayed by the grower on June 19 with ½ pound of actual parathion per acre. At the time this application was made there were from two to eight eggs at or on the base of each plant. It was estimated that this field would have been a total loss if this application had not been made. An infestation of

Table 3.—Effectiveness of soil treatments and foliage applications of several organic insecticides for control of the maggot complex in spinach, 1955–56.

Material[a] and Per Cent Active Ingredient	Pounds of Toxicant per Acre	Per Cent of Plants Infested	Per Cent Control
Soil Treatments, 1955			
Dieldrin Em, 15	5	20[b]	45
Heptachlor Em, 23.4	5	25[b]	32
Untreated		37	
Foliage Applications, 1955			
Malathion WP, 25	0.75	34	37
Malathion WP, 25 plus Yeast protein	0.75+ 1.0	24[b]	57
Malathion Gr, 10	0.75	11[b]	80
Parathion WP, 15	0.25	22[b]	59
Dieldrin WP, 50	0.50	17[b]	68
Dieldrin WP, 50 (1st spray)	0.50		
Malathion WP, 25 (4 sprays)	0.75	20[b]	62
Diazinon WP, 25	0.50	19[b]	65
Phosdrin, Em (10.4 lb./gal.)	0.30	44	18
Dow ET-57 WP, 25	0.75	20[b]	64
Untreated		54	
Foliage Applications, 1956			
Dieldrin Em, 15	0.5	9[b]	68
Dieldrin Gr. 5	.5	0.3[b]	99
Parathion Em, 25	.5	11[b]	59
Parathion Gr, 2.5	.5	3[b]	89
Malathion Gr, 5	1.0	6[b]	78
Heptachlor Gr 2.5	.5	3[b]	90
Guthion Em, 23	1.0	6[b]	78
Dow ET-57 Em, 50	1.0	16	41
Thiodan WP, 25	.5	20	29
Dipterex WS, 25	1.0	17	40
Untreated		28	

[a] Em, emulsion; WP, wettable powder; Gr, granulated; WS, water soluble.
[b] These means differ significantly from the control at the 5% level based on Duncan's (1955) multiple range test.

28% developed in this field following the heavy parathion treatment.

RESIDUE STUDIES.—Residue studies have been conducted in conjunction with the various treatments during the 3-year program. Table 4 presents the data from the residue studies during 1954 in which both chemical and bioassay determinations were made in cooperation with the National Canners Association. Spinach from the 1954 plots treated with four applications of parathion, malathion, DDT and lindane and sampled 1 day after treat-

Table 4.—Insecticide residues[a] on samples of fresh and canned spinach taken from plots having four applications of wettable powders in 1954.

Material and Per Cent Active Ingredient	Pounds of Actual Toxicant per Acre	Interval (Days)	Residue in P.P.M.			
			Bioassay		Chemical	
			Fresh	Canned	Fresh	Canned
Parathion, 15	0.15	1	6.8	2.8	6.8	1.7
Parathion, 15	.15	7	0.05	<0.03	0.1	0.06
Lindane, 25	.25	1	9.9	0.06	5.4	0.3
Lindane, 25	.25	7	0.03	<0.03	<0.05	<0.05
DDT, 75	1.0	1	74	5.0	71	18
DDT, 75	1.0	7	1.1	0.4	3.8	1.4
Malathion, 25	.75	1	12	4.8	14	<0.5
Malathion, 25	.75	7	0.3	<0.3	<0.5	<0.5

[a] Residue analyses made by C. J. Tressler of the National Canners Association.

Table 5.—Insecticide residues on fresh and canned spinach samples taken from plots having foliage and soil treatments during 1955.[a]

Material and Per Cent Active Ingredient	Pounds of Actual Toxicant per Acre	Residue in P.P.M.			
		3 Days		7 Days	
		Fresh	Canned	Fresh	Canned
Foliage Treatments (5 applications)					
Malathion Em, 57	0.75	<1.0	—	<1.0	—
Malathion Gr, 10	.75	<1.0	—	<1.0	—
Parathion WP, 15	.25	1.2	0.24	0.05	0.03
Dieldrin WP, 50	.50	3.2	0.05	1.2	<0.03
Diazinon WP, 25	.50	1.2	no sample	0.11	<0.09
Phosdrin Em, (10.4 lb./gal.)	.33	<0.05	—	<0.05	—
Dow ET-57 WP, 25	.75	2.6	<0.11	<0.11	—
Untreated		0		0	
Soil Treatment[b] (1 application)					
Heptachlor Em, 23	5	<0.07	—	<0.07	—
Dieldrin Em, 15	5	0.06	<0.03	<0.03	<0.03

[a] Residue analyses made by C. J. Tressler of the National Canners Association.
[b] Interval was 65 days between treatment and taking of samples.

ment contained residues above the allowable tolerances for these materials. Corresponding samples taken 1 day after application and canned were all below the established residue tolerance with the exception of the spinach receiving the parathion treatment. The parathion treated and canned spinach had 2.8 p.p.m. residue according to the bioassay determination and 1.7 p.p.m. by chemical analysis. Samples of fresh and canned spinach taken 7 days after treatment were all below the minimum tolerance for these four materials.

Table 5 presents the data from house fly bioassay studies of fresh and canned spinach from the 1955 plots. Fresh spinach treated with malathion and Phosdrin harvested 3 days after the last application did not contain measurable amounts of insecticidal residue. Residues in spinach from all of the plots harvested 3 days after the last application and canned were below the established tolerance in those cases where a tolerance has been established. With the exception of dieldrin all residues in fresh spinach were below the established tolerance 7 days after treatment. The only detectable residue on spinach grown on soil treated previous to planting was in the dieldrin plots.

In 1956 residue analyses were made on samples taken from the malathion and dieldrin plots. There was no detectable malathion in any of the samples. These included samples taken 30 minutes after the application of granulated malathion at 1 pound per acre.

The results of bioassay studies of fresh spinach harvested from the dieldrin treated plots during 1956 are presented in table 6. The dieldrin residue on spinach from spray treated plots was generally three to four times higher than from spinach where granulated materials at comparable dosages were applied. Additional residue studies are in progress.

SUMMARY AND CONCLUSIONS.—The presence of maggots in the expanding buds of spinach at the time of harvest has presented a serious contamination problem for the processing industry in several states. A 3-year investigation was initiated to study the biology and control of the maggot complex (budworms) associated with the buds of spinach.

Eleven species of flies representing six families were reared from infested spinach. A single species of *Drosophila, Scaptomyza graminum* Fallen, was responsible for 71% of the population. The remaining population was composed primarily of the seed-corn maggot (*Hylemya cilicrura* (Rond.)), the false stable fly (*Muscina stabulans* (Fall.)) and *Muscina assimilis* (Fall.).

Foliage treatments with the chlorinated hydrocarbon insecticides were generally more effective than the phosphate materials. Soil treatments reduced the population a maximum of 45%, which was not sufficient to be considered practical. Foliage applications with granulated carriers were more effective than conventional spray treatments. The granulated materials were applied to the dry foliage and the granules funneled into the crown area where the infestation was concentrated.

Residues in canned spinach sampled 3 days after treatment were generally absent or below the established tolerances for the materials included in this study. Dieldrin residues on spinach treated with sprays were four to six times greater than on spinach treated with granulated dieldrin at the same rate. Both the performance data and the residue studies indicated that granulated dieldrin and parathion were the most promising materials for control of the maggot complex on spinach.

Table 6.—Residues on fresh spinach treated with foliage application of dieldrin at 0.5 lb. per acre during 1956.[a]

Formulation and Per Cent Active Ingredient	Number of Applications	Interval	Residue (P.P.M.)
Emulsion, 15	4	3 days	2.6
Granulated, 5	4	3 days	3.4
Untreated			0
Emulsion, 15	1	2 days	4.4
Granulated, 5	1	2 days	1.0
Emulsion, 15	2	30 minutes	14.0
Emulsion, 15	2	4 days	2.7
Granulated, 5	2	30 minutes	4.0
Granulated, 5	2	4 days	1.0

[a] Residue analyses made by the Shell Chemical Corporation.

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Types and Seasonal Incidence of Stink Bug Injury to Peaches[1]

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For many years Ohio peach insect investigations have been devoted to the control of the oriental fruit moth, the plum curculio, orchard mites and peach tree borers. As effective controls for these species were gradually developed, it was observed that there was considerable insect injury to fruit which was not associated with these pests. Exploratory studies, based upon work done in other states, revealed that much of this injury was due to various species of stink bugs and the tarnished plant bug. One of the most obvious types of injury produced by these insects was a severe distortion or deformation of the fruit which has been commonly known as "cat-facing." Insects which produce fruit deformity have come to be known as "cat-facing insects." The term "cat-facing insects" involves a complex of species including certain plant bugs, stink bugs and more diverse types such as the plum curculio. Cat-facing injury in 1954 in some orchards accounted for the destruction of from 18 to 36% of the total crop even though a complete insecticidal spraying program was followed. In 1954, following a survey of 60 commercial peach orchards, it was estimated that cat-facing insects destroyed a portion of the Ohio peach crop valued at approximately $250,000.

In 1953, cage experiments were begun in Ohio to study the complex of insect species involved in this problem. These investigations were continued through the 1956 season. The objectives of this work were: (1) To determine the insect species responsible for producing cat-facing and other types of injury; (2) To determine the types of injury produced by the various species; (3) To correlate the seasonal incidence of injury with the seasonal development of the peach fruit. The results of the different phases of the problem will be reported in a series of publications of which this paper forms a part. This report deals only with the investigational phases involving various species of Pentatomidae.

Serious injury to peaches by stink bugs was first brought to the attention of entomologists in 1911 when the green stink bug, *Acrosternum hilare* (Say), caused severe losses in orchards along the Marblehead peninsular regions of Ohio near Lake Erie (Whitmarsh 1917). In 1928 Porter *et al.* on the basis of cage tests and field observations in Indiana and Illinois, reported that cat-facing was produced by four species of stink bugs, the brown stink bug (*Euschistus servus* (Say)), the northern brown stink bug (*E. euschistoides* (Vollenhaven)),[2] the dusky stink bug (*E. tristigmus* (Say)) and the one-spot stink bug (*E. variolarius* (P. de B.)), as well as by the tarnished plant bug (*Lygus lineolaris* (P. de B.)) and the plum curculio (*Conotrachelus nenuphar* (Hbst.)). The list of injurious species was further supplemented by Rice in 1937 who stated that the stink bug, *Hymenarcys nervosa* (Say), produced cat-faced peaches in one of his experiments. Woodside (1946) confirmed the work of Porter *et al.* and added the red-shouldered stink bug, *Thyanta custator* Fab., to the list of cat-facing pentatomids. Snapp (1954) reported that the green stink bug and the southern green stink bug, *Nezara viridula* (L.), sometimes caused serious damage to peaches in Georgia orchards.

METHODS.—Exploratory studies were conducted in 1953 and 1954 to determine which species of stink bugs produced injury to peaches. The species used in this phase of the investigation were selected by their association with peaches. Several large peach orchards were jarred throughout the season to determine the occurrence and seasonal abundance of various stink bug species. Species which were found to be abundant or moderately abundant on peach trees were subsequently used in cage tests.

In 1955 and 1956 each of the species implicated in the previous tests was caged with peach blossoms or fruit for a 1- to 4-day period each week from bloom until harvest. Observations on feeding habits were made daily during the exposure period, and when injury was produced the insect was removed to prevent excessive injury and consequent fruit dropping. Dead insects were replaced with live ones when necessary. Observations on the type of injury produced by each species were made at weekly intervals throughout the season.

In 1953 and 1954 only stink bugs which were jarred from peach trees were employed in the cat-facing studies. In 1955 and 1956 a more adequate supply of stink bugs was obtained by collecting the insects in early April from southern Ohio on peach and common mullein, *Verbascum thapsus* L. Many specimens of *Acrosternum hilare* and *Euschistus servus* were received through the courtesy of Oliver Snapp, Entomology Research Division, Agricultural Research Service, Fort Valley, Ga., and Josef Knull, Department of Zoology and Entomology, Ohio State University, Columbus, Ohio. Between exposure periods the stink bugs were removed to an insectary where they were allowed to feed on green string beans.

In order to correlate insect injury with the seasonal development of the peach, 100 Elberta fruits were sampled at random each week from trees in the experimental orchard. Half of these fruits were measured for cheek diameter and half for suture diameter. The volume of the fruits was also determined each week by their displacement of ethyl alcohol in a graduated cylinder. Volume was considered a better criterion of growth than diameter because it gave an estimate of growth in all directions.

The cage employed (fig. 1) was a modification of the bivalve type of insect feeding cage described by Wilde (1951). The cylindrical cages, 12 inches long and 6½ inches in diameter, consisted of two halves, hinged on one side with scrap leather and equipped on the other with metal fasteners. The seams were tightly sealed when the cage was closed by lining the connecting edges with sponge-rubber weather stripping. One side of the cage, which would normally be upwards when in position on the tree, was covered with transparent celluloid and the

[1] This project was supported cooperatively by the Ohio Agricultural Experiment Station and the Section of Fruit Insect Investigations, Entomology Research Division, Agricultural Research Service, U. S. Department of Agriculture. Accepted for publication March 27, 1957.

[2] This species is now considered a subspecies of *Euschistus servus* (Say).

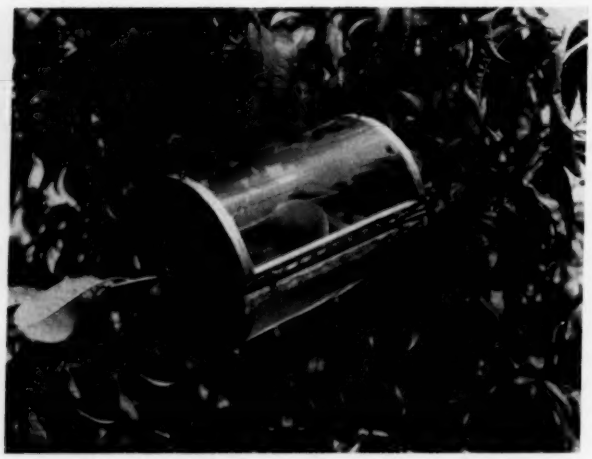

FIG. 1.—Bivalve type of insect feeding cage used in stink bug investigations.

other side was covered with a 32-mesh plastic screen. An insertion valve similar to that used by Wilde was used to introduce insects, but it was usually necessary to open the cage to remove insects.

SPECIES OF STINK BUGS PRODUCING INJURY TO PEACHES.—During the course of this investigation 14 species of stink bugs were used in 654 cage tests to determine which species were responsible for various types of injury to peaches. Twelve species of stink bugs which were moderately abundant on peaches were used in cage tests. In addition, two species, *Banasa dimidiata* Say and *Thyanta calceata* Say, which do not ordinarily occur on peach were also included. The species employed and the species producing injury to peaches are listed in table 1. All of the species were initially identified by R. I. Sailer, Section of Insect Identification and Parasite Introduction, Entomology Research Division, Agricultural Research Service, U. S. Department of Agriculture. It may be noted that only six of the species are of any economic importance and some of these are economically important only in certain areas of the state. The remaining species are relatively uncommon or if common do not feed readily upon peaches.

The predaceous forms, *Podisus maculiventris* (Say) and *Podisus placidus* Uhler, although fairly common in peach orchards could not be induced to feed upon caged peaches. Another species, *Brochymoena quadripustulata* Fab., feeds primarily on twigs and limbs but was induced to feed upon the fruit when caged. The comparatively few peaches injured by this species, even under forced feeding conditions, would indicate that an occasional fruit may be injured under natural conditions, particularly by nymphs, but that the incidence of injury would be extremely low.

TYPES OF INJURY PRODUCED BY STINK BUGS.—Although there was some variation in the injuries produced by different species of stink bugs, these injuries could be classified into several distinct groups.

Blossom or fruit drop.—Blossoms or immature fruit less than 13 millimeters in diameter often drop as a result of stink bug feeding. This type of injury is normally of no significance but may reduce the yield in seasons when the blossom set is very light.

Cat-facing or deformation injury.—Any injury which resulted in fruit deformation was classified as cat-facing injury (fig. 2, A). In most cases the injury consisted of deep, depressed scars, devoid of pubescence except for small patches of grayish or brownish fuzz. The scars usually had a mass of gum in their centers. There was considerable variation in the size of the scars and the larger ones were undoubtedly a coalescence of several smaller scars. Atypical cat-facing included slight to deep depressions which were neither scarred nor devoid of fuzz but sometimes had a concentration of red pigment in the area of deepest indentation. This type of deformation is sometimes known as "dimpling" or "puckering."

Scarred injury.—This injury was somewhat similar to that described above and differs only in that there is no fruit deformation. Typical injury consisted of a brown, corky, scarred area, devoid of fuzz except for small patches of grayish or brownish fuzz scattered over the affected area. The scars usually had a mass of gum in their centers and in some instances there was a concentration of reddish pigment around the edges.

Gummosis injury.—This condition is characterized by an exudation of gum after the epidermal tissues are punctured by feeding insects. As previously mentioned, gummosis is usually associated with both cat-facing and scarring injury. However, it may occur alone as a response to feeding as shown by figure 3. The fruit exudes gum in droplets or in strings as is shown in figure 2, B. During rains, when the fruit is unprotected by caging, the gum sometimes softens and spreads out over the surface of the fruit. Quite often the gummy exudate assumes a blackish color which is believed to be due to the development of a sooty fungus. In many cases, particularly when gummosis is produced late in the season, the gum droplets harden and if they are removed the fruit is normal in appearance.

Some fruits showing gummosis also had from slight to severe flesh injury. In these cases there were brownish areas of irregular shape, which extended from 5 to 16 mm. beneath the surface of the fruit. Sometimes, in the center of the injured flesh area, there was a hollow area, 2 to 3 mm. wide, filled with clear gum.

Water-soaked injury.—Fruit showing this condition had dark green areas, sometimes depressed, which had a water-soaked appearance (fig. 2, C). In many cases this was associated with gummosis. When peaches showing this type of injury were sliced, flesh damage consisting of brownish, irregular areas which extended from 5 to 16 mm. beneath the epidermis was also evident (fig. 2, D). In some instances water-soaked injury was scarcely visible externally, but the flesh injury was so severe that the fruit was unacceptable for consumption.

SEASONAL INCIDENCE OF VARIOUS TYPES OF INJURY.—In cage tests with five of the more important species of stink bugs, which are designated by footnote reference in table 1, it was noted that the peach responded in several ways to insect feeding at different stages of development. The stage of fruit development had a much greater influence on the type of injury produced than the species of stink bug initiating the injury. An example of this is that all five species produced cat-facing at the shuck-fall stage, but it was impossible to detect differences in the cat-facing produced by four of these species. Gummosis was produced by all five species 60 days after bloom, yet

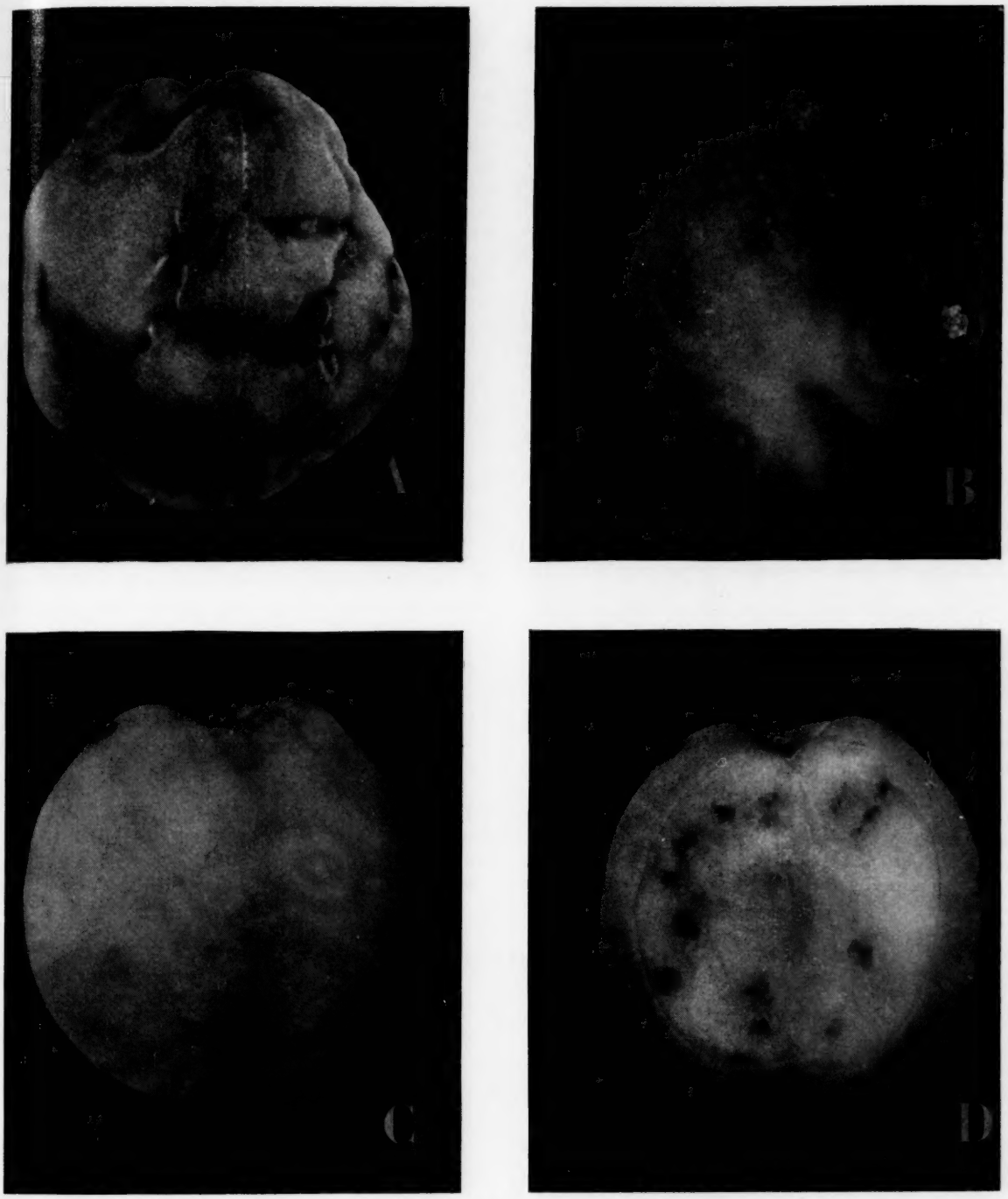

FIG. 2.—Elberta peaches showing various types of injury produced by the green stink bug, *Acrosternum hilare* (Say). A.—Cat-facing injury. B.—Gummosis injury. C.—Water-soaked injury. D.—Fruit shown in (C) sliced to show flesh injury beneath water-soaked areas.

the injury produced by different species was identical. The seasonal incidence of various types of injury produced by different species of stink bugs is summarized in table 1.

A graphic correlation between the seasonal incidence of injury produced by two species of stink bugs and the seasonal development of the fruit is shown in figure 3. The diameter and the volume of the fruit are plotted against the number of days after bloom in the lower graph. In the upper graph the seasonal incidence of various types of injury produced by *Acrosternum hilare* is plotted against the number of days after bloom. The number of fruits injured refers to the actual number of fruits which were damaged in four cage replicates each week. An average of 10.3 fruits were exposed in four cage replicates each week. A similar correlation is shown in the middle section of figure 3 for *Euschistus variolarius*. Since other species produced injuries of almost identical types and

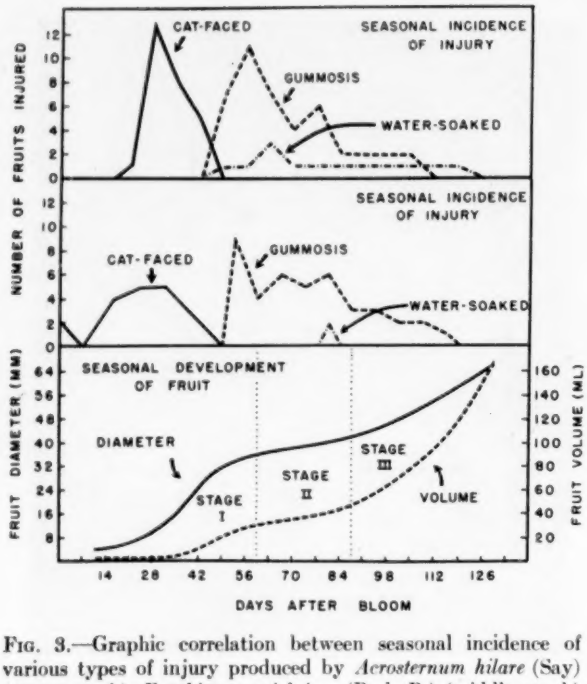

FIG. 3.—Graphic correlation between seasonal incidence of various types of injury produced by *Acrosternum hilare* (Say) (upper graph), *Euschistus variolairus* (P. de B.) (middle graph) and the seasonal development of the fruit (lower graph).

the seasonal incidence was nearly the same, graphs for *Euschistus tristigmus*, *E. variolarius* and *Thyanta custator* have not been shown.

Cat-facing was produced from bloom up to 49 days after bloom. In general, the earlier in the season the fruit was injured the more severe the deformity.

Scarring without deformation was rarely encountered as a result of stink bug feeding but quite frequently followed injury by various species of plant bugs. In cage tests with four species of stink bugs (*Acrosternum hilare*, *Euschistus servus*, *E. tristigmus* and *E. variolarius*), scarring occurred following feeding from 42 to 56 days after bloom.

Gummosis injury alone, as shown in figure 3, occurred from 42 to 115 days after bloom and usually reached a peak during the final portion of developmental Stage I or in the early portions of Stage II. In the first developmental stage of the peach, gummosis was severe, often occurring in long strings and sometimes forming small masses of gummy exudate in the bottom of the cages. During the second developmental period not only did fewer peaches respond to injury by exuding gum but the amount of gum exuded was very slight as compared with Stage I. The incidence of gummosis was comparatively low in Stage III, and the fruit no longer exuded gum when injured within 2 weeks of harvest.

DISCUSSION.—Since various types of injury were produced at different stages of peach development, the different responses might be explained by chemical, physical and histological changes in the fruit during its development.

A number of horticultural workers (Lott 1942, Lilleland 1933, Blake 1926) have noted a cyclic growth in the peach which may be divided into three developmental stages. The first period was characterized by a rapid increase in size and fresh weight of flesh, stone and kernel. In the second growth stage the flesh, stone and kernel increased at a relatively slow rate and the rate of accumulation of all constituents in the flesh and kernel was reduced. The third growth period was characterized by an accelerating increase in size and dry matter of the flesh, with a particularly rapid increase in sucrose during the last 11 days. The exact transition dates from one period to another are not well defined; therefore, only approximate transition dates are shown in figure 3.

It may be noted in figure 3 that cat-facing or fruit deformation with gummosis occurred only in developmental Stage I. A possible clue may be found in the works of Addoms *et al.* (1930) who state that the early growth of the ovary after fertilization is associated with rapid cell

Table 1.—Importance of stink bugs and types of injury produced in peaches at different stages of development.

SPECIES	ECONOMIC IMPORTANCE	NUMBER OF CAGE TESTS	SEASONAL INCIDENCE OF INJURY EXPRESSED IN NUMBER OF DAYS AFTER BLOOM			
			Cat-Faced and Gummy	Scarred and Gummy	Gummy Only	Water-Soaked and Gummy
Acrosternum hilare (Say)[a]	In northern Onio	93	21–45		45–105	45–119
Banasa dimidiata Say	None	6			51	
Brochymoena quadripustulata Fab.	None	32			42–77	
Dendrocoris humeralis (Uhler)	None	8				
Euschistus servus servus (Say)[a]	In southern Ohio	91	0–42	42–56	56–98	77
E. servus euschistoides (Voll.)[a]	In some localities	96	0–28		49–112	
E. tristigmus (Say)[a]	Important	113	0–28	49	49–112	
E. variolarius (P. de B.)[a]	Important	93	0–49	42–49	49–115	80
Holcostethus limbolarius (Stal.)	None	25	30			
Hymenarcys nervosa (Say)	None	15				
Podisus maculiventris Say	Predaceous	13				
Podisus placidus Uhler	Predaceous	5				
Thyanta calceata Say	None	2				
Thyanta custator Fab.[a]	In some localities	57	0–30		42–91	
Trichopepla semivittata Say	None	5				

[a] Species used in cage tests from bloom to harvest in 1955 and 1956.

division. Within the third week after bloom however, the rate of division decreases until the 30th day when it practically ceases. Thereafter increase in size is effected largely by the enlargement of cells already formed. It might be theorized that early stink bug feeding interferes with normal cell division with resultant cat-facing and scarring. However, since stink bug cat-facing and scarring injury occurs from bloom to 56 days after bloom, with the final damage occurring about 3 weeks after the conclusion of cell division, it would appear that this would explain only the fact that the earlier an injury is caused the more severe the fruit deformation.

A histological study of the peach conducted by Dorsey & Potter (1932) provides a more likely explanation. These studies showed that below the epidermis there is a layer of hypodermal cells from four to eight cells in depth, which are tightly packed and fairly thick-walled. Kessler (1955) points out that in certain varieties of apples this hypodermal tissue reverts to a meristematic condition and produces a cork tissue commonly referred to as "russet." He further states that if the epidermis of a fruit is injured in some way, while the fruit is growing, it will often develop a protective layer of corky tissue over the injured surface.

It appears that this mechanism is also operative in peaches and may explain scar formation following exposure to stink bug feeding. It is believed that the hypodermal cells, located just beneath the epidermis, are potentially meristematic and only become actively meristematic when stimulated by some abnormal factor such as mechanical or insect feeding injury. In the present work, meristematic scars have resulted following early season feeding by stink bugs, plant bugs, plum curculio, red-banded leaf roller and oriental fruit moth. Since caged insects fed upon peaches in all stages of development and meristematic scars occurred as a response to feeding only from bloom to 56 days after bloom (table 1), it must be assumed that the hypodermal cells lose their meristematic potentiality at about 56 days after bloom.

The reasons for differences in response of gummosis to injuries at various stages of development are not clearly understood. It is well known that the exudation of gum acts as a protective mechanism against destructive organisms, yet the reasons for this reaction in stone fruits have not been investigated. In a study of the structure of the skin, Dorsey & Potter (1932) reported that in the early stages of development only the epidermal layer protects the young fruit, but as growth and enlargement proceed, the deeper layers supplement the epidermis as protective tissues. It is quite possible that during most of Stage I the epidermis is the only functional protective tissue, and therefore wounds caused by insect feeding heal slowly and allow copious gum flow. However, as the fruit matures in Stages II and III, additional protection is provided by the underlying layers and the wounds heal more rapidly with a consequent reduction in gum exudation. Perhaps there are other factors involved, such as the degree of tension in the cells just below the epidermis, although little is known in this regard.

Water-soaked injury apparently involves tissue mechanisms similar to those involved in the cat-facing response although it varies considerably in external appearance. When this type of injury is produced early in the season the damaged area may be depressed, but no corky tissue is produced. The hypodermal cells in the affected area apparently retain chlorophyll for an indefinite period while in the normal cells surrounding the area the chlorophyll gradually disappears with the maturation of the fruit.

SUMMARY.—Cage tests were conducted from 1953 to 1956 with 14 species of stink bugs to determine the types of injury produced in peaches. The different types of injury produced were: (1) Blossom and fruit drop, (2) Cat-facing or deformation injury, (3) Scarred injury, (4) Gummosis injury and (5) Water-soaked injury. Two species, *Euschistus servus* (Say) and *E. variolarius* (P. de B.), produced all five types of injury. Other species producing cat-facing were *Acrosternum hilare* (Say), *E. tristigmus* (Say), *Holcostethus limbolarius* (Stal.) and *Thyanta custator* Fab. All of the above species of *Euschistus* produced scarred injury. Two species, *Brochymoena quadripustulata* Fab. and *Banasa dimidiata* Say, produced only gummosis injury.

In one phase of the investigation the injury produced by five of the more important species was studied from bloom until harvest. It was noted that the injured fruits responded differently to insect feeding injury at different stages of development. Cat-facing was produced from bloom to 49 days after bloom while scarring, which is considered to be a mild form of cat-facing without deformation, occurred from 42 to 56 days after bloom. Gummosis was the most prevalent type of injury and occurred from 42 to 115 days after bloom. Water-soaked injury was produced when peaches were exposed to feeding from 45 to 119 days after bloom.

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Residual Effectiveness of Insecticide Formulations in Killing Resistant and Non-Resistant German Cockroaches[1]

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Laake (1955) has reported on the results obtained from the use of a number of insecticidal formulations in control of chlordane-resistant German cockroaches, and non-resistant American and oriental cockroaches. Although Chlorthion was found to be highly effective for control of resistant German cockroaches, it was found to be objectionable to operators and to personnel in treated buildings. Certain other formulations were found to be effective and not objectionable but, all things considered, Malrin was reported to be the safest material for controlling resistant German cockroaches; whereas mixtures of malathion and dieldrin gave the best results when the American, the oriental, and resistant German cockroaches were all present.

The purpose of the tests reported here was to determine the effectiveness of a number of insecticidal formulations in killing resistant and nonresistant German cockroaches after the residual deposits had aged for different periods of time. Originally it was planned to test the deposits after they had aged for 1, 8, 15, 21, 30, and 60 days, but later it appeared unnecessary to test those that had aged for periods less than 21 days. The resistant strain of the German cockroach used in these tests originally came from Corpus Christi, Texas, and was found by Grayson (1954) to be very highly resistant to chlordane. This strain was periodically exposed to masonite tiers treated with 2 to 5% chlordane in an attempt to maintain the level of resistance.

PROCEDURE.—The cockroaches were reared and maintained at 30° C. and 55% relative humidity prior to their use in testing. Breeders were kept in 1-gallon, glass battery jars, about 60 pairs per jar, and the nymphs produced were removed to 4-gallon aquaria. Care was exercised to prevent any of the cultures from becoming too crowded. Adult cockroaches within known age limits were used in the tests; these were obtained by removing all adults from the aquaria cultures at approximately weekly intervals.

Three different types of surfaces were employed in an attempt to simulate conditions existing under practical circumstances involving infestations of cockroaches. Seasoned, unpainted wood was selected as a rather absorptive surface, tempered masonite as a moderately absorptive surface, and galvanized sheet metal as a rather non-absorptive surface. These were cut into panels, 4½ by 4½ inches in size, and with the corners trimmed to permit fitting into 1-gallon battery jars. The panels were treated by dipping them in the desired insecticide formulation, allowing them to dry, and then suspending them from the joists of a service building until they had aged for the proper length of time. All surfaces of the panels were exposed to air movements by suspending them in vertical racks with spaces between panels.

The testing procedure consisted of placing 20 female German cockroaches in each battery jar containing three treated panels. Each test was replicated four times, making a total of 80 cockroaches for each test (except in one series with a total of 70). Mortality readings were taken at the end of 1, 3, and 6 days following exposure of the cockroaches to the treated panels.

MATERIALS.—Sixteen formulations involving nine different materials were included in the experiment. The materials were as follows: technical chlordane; Dieldrex 15; malathion emulsifiable liquid (57%), premium and regular grades; Diazinon 25E; technical Dipterex; Am. Cyanamid 4124, 25% emulsifiable liquid; Malrin; technical allethrin; and pyrethrum, 10% in oil. A refined kerosene was used in preparing the oil base formulations.

Am. Cyanamid 4124 is neither registered nor recommended for indoor insect control as its odor would be objectional. Individually, but not necessarily in the combinations shown, the remaining insecticides are registered for control of indoor populations of cockroaches. The label for Diazinon, however, restricts its use in dwellings to pest control operators only.

RESULTS.—The results are shown in table 1. A total of 16 formulations were tested on tempered masonite panels after the deposits had aged for 21, 30, and 60 days; however only the data from the 21- and 60-day periods are shown. Six of the more promising materials were further tested on galvanized sheet metal and wood panels after the deposits had aged for 21 and 30 days. The most effective of the formulations tested was Am. Cyanamid 4124 which gave complete kill of resistant and non-resistant cockroaches on all surfaces within the first day following exposure to the treated panels. Complete kill within 3 days following exposure was obtained with Diazinon on masonite and wood, but only 33 to 54% were killed after an exposure of 6 days to this material on galvanized sheet metal. Five different formulations of malathion, two of which contained allethrin, and Malrin gave from 98 to 100% kill of cockroaches on masonite panels after 6 days of exposure, while almost complete kill was obtained at the end of 3 days of exposure to metal and wood panels

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Table 1.—Residual effectiveness of insecticidal formulations in killing females of German cockroaches from resistant and non-resistant strains.

Formulation[a] and Per Cent of Active Ingredient	Per Cent Mortality of Strains at End of (Days): Group A[b] Resistant 1	Group A[b] Resistant 3	Group A[b] Resistant 6	Group A[b] Non-Resistant 1	Group A[b] Non-Resistant 3	Group A[b] Non-Resistant 6	Group B[c] Resistant 1	Group B[c] Resistant 3	Group B[c] Resistant 6	Group B[c] Non-Resistant 1	Group B[c] Non-Resistant 3	Group B[c] Non-Resistant 6
On tempered masonite panels												
Chlordane, 2	0	0	0	19	40	57	1	4	4	28	66	74
Chlordane, 2+allethrin, 0.1	1	1	9	46	63	79	0	4	9	19	46	76
Chlordane, 2+pyrethrum, 0.1	15	23	29	41	55	75	10	18	25	16	48	90
Dieldrin, 0.5	0	6	11	30	60	73	1	4	10	43	71	80
Malathion, 2	99	100	100	63	99	100	89	98	100	50	90	100
Malathion, 2 (premium grade emulsion)	96	100	100	83	100	100	95	100	100	68	95	100
Malathion, 2 (regular grade emulsion)	96	100	100	66	89	100	85	96	100	54	84	100
Malathion, 2+allethrin, 0.2	97	99	100	49	99	100	91	98	100	40	89	98
Malathion, 2+allethrin, 0.1	97	100	100	43	90	100	88	95	100	61	94	99
Diazinon, 0.5 (emulsion)	100	100	100	93	100	100	98	100	100	88	100	100
Dipterex, 1	54	99	100	59	99	100	16	54	84	6	45	79
Dipterex, 1 (water solution)	31	64	94	44	65	86	18	33	54	38	50	68
Am. Cyanamid 4124, 2 (emulsion)	100	100	100	100	100	100	100	100	100	100	100	100
Am. Cyanamid 4124, 2+ allethrin, 0.1 (emulsion)	100	100	100	100	100	100	100	100	100	100	100	100
Malrin, 4	89	100	100	47	96	100	90	95	99	34	85	99
Allethrin, 0.1	0	1	3	1	11	49	0	1	1	0	1	16
On wood panels												
Chlordane, 2	10	39	74	60	76	99	9	64	95	63	83	94
Dieldrin, 0.5	28	89	99	63	79	95	15	84	98	55	81	95
Diazinon, 0.5	98	100	100	94	100	100	91	100	100	94	100	100
Am. Cyanamid 4124, 2 (emulsion)	100	100	100	100	100	100	100	100	100	100	100	100
Malathion, 2+allethrin, 0.2	99	100	100	86	100	100	76	100	100	71	100	100
Malathion, 2+allethrin, 0.1	98	100	100	73	100	100	84	99	100	79	99	100
On galvanized sheet metal panels												
Chlordane, 2	6	25	39	11	31	60	0	10	26	3	50	73
Dieldrin, 0.5	3	9	28	33	50	78	0	0	1	1	41	54
Diazinon, 0.5	10	24	33	4	29	41	0	26	33	10	44	54
Am. Cyanamid 4124, 2 (emulsion)	100	100	100	100	100	100	100	100	100	100	100	100
Malathion, 2+allethrin, 0.2	94	100	100	85	100	100	95	100	100	88	100	100
Malathion, 2+allethrin, 0.1	90	99	100	73	99	100	89	100	100	78	100	100

[a] The formulations were oil base unless otherwise indicated.
[b] All treated panels had aged for 21 days before cockroaches were placed on them.
[c] The tempered masonite panels had aged for 60 days, and the wood and galvanized sheet metal panels had aged for 30 days, when cockroaches were placed on them.

treated with malathion plus allethrin. Dipterex in oil was more effective than in water solution, but in either case considerably less cockroaches were killed after the deposits had aged for 60 days as compared with 21 days. Very low percentages of kill of resistant roaches were obtained from chlordane, with or without allethrin or pyrethrum, and dieldrin on masonite panels; and only fair kill of non-resistant roaches was obtained with these formulations on masonite panels. Allethrin on masonite panels gave almost no kill except of non-resistant cockroaches exposed to deposits which had aged for the shortest period of time.

SUMMARY.—The residual effectiveness of 16 insecticidal formulations applied to tempered masonite panels in killing female German cockroaches from chlordane-resistant and non-resistant strains was determined. The panels were dipped in the insecticidal formulations and the deposits allowed to age for periods of 21, 30, and 60 days before placing the cockroaches on them. Six formulations were further tested on wood and galvanized sheet metal panels after the deposits had aged for 21 and 30 days.

The most effective of the formulations tested was Am. Cyanamid 4124 which gave complete kill on all surfaces in 1 day. Diazinon gave complete kill in 3 days on masonite and wood, but it gave very poor results on galvanized sheet metal. Several formulations of malathion, with and without allethrin, and Malrin gave good control in 6 days. Only fair control was obtained with Dipterex, while chlordane and dieldrin gave unsatisfactory control.

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The Role of Dietary Fatty Acids in the Development of the Pink Bollworm[1]

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It was reported previously that the pink bollworm, *Pectinophora gossypiella* (Saund.), has a dietary requirement for fat (Vanderzant & Reiser 1956b). When corn oil was added to a basal fat-free medium, normal adults emerged from their pupal cases, whereas no moths emerged when it was omitted. Subsequently it was found that linoleic acid would replace corn oil (Vanderzant *et al.* 1956). In this paper the effects of various fatty acids and natural fats upon the growth and development of the pink bollworm is discussed.

Fraenkel & Blewett (1945) reported that moths of the genus *Ephestia* did not develop normally unless wheat germ oil was added to the diet. In the absence of wheat germ oil growth was slow, the mortality high, and the moths failed to emerge, and with suboptimal amounts of this oil moths emerged with their wings lacking in scales. Subsequently, they found the active substance to be linoleic acid. Linolenic acid had approximately the same effect, and oleic acid had no activity (Fraenkel & Blewett 1946). Arachidonic acid had no effect on the development of the wings, but it accelerated growth of the larvae (Fraenkel & Blewett 1947).

Linoleic, linolenic, and arachidonic acids and other polyunsaturated fatty acids are known to be essential growth factors for the rat, mouse, chicken, dog, and probably other vertebrates. Proof of this requirement often is difficult to obtain, since the young have essential fatty acids stored in their tissues at birth, and fat-free diets are difficult to design for some animals.

No insect other than those in the genus *Ephestia* and the pink bollworm have been found to require fats in the diet, although there are several reports that fats have a beneficial effect on the growth of other insects (House 1956, Scoggin & Tauber 1950, Trager 1953). Unfortunately, in many of the earlier studies sterols as well as fatty acids were present in the materials tested. Hence, it cannot be determined whether the positive effects were caused by the fatty acids or by the sterols, since the latter have been shown to be a dietary requirement for all insects that have been studied. Failure to observe the need of other species for fat may be due to the presence of significant amounts in the test diets and the general lack of nutritional data on the development of insects through adulthood and oviposition.

METHODS.—The procedures and equipment for preparing aseptic eggs, inoculation of vials, incubation, and observations have been reported previously (Vanderzant & Reiser 1956a). Several modifications of the original basal casein medium were made, and the new medium is given at the top of the next column.

The preparation of the medium was similar to that previously reported (Vanderzant & Reiser 1956b). To test the growth effects of various lipides, the corn oil was omitted and the lipide was then added and mixed thoroughly. Care was taken not to add too much test lipide to the medium lest the caloric balance be seriously upset.

The evaluation of results was similar to that in the previous papers. However, the types of emergence were divided into three groups: (a) no emergence, (b) partial emergence in which the pupal case was broken open and either the head or tail was free but the wings were still attached, and (c) complete emergence with well-formed moths.

Constituent	*Per 100 Grams of Diet* *Grams*
Casein, "vitamin-free" (Nutritional Biochemicals Company)	5.0
L-Cystine	0.1
Glycine	0.15
Sucrose, C.P.	5.0
Corn oil (Mazola)	0.25
Cholesterol, U.S.P.	0.05
Salt mixture, Wesson's	1.2
Choline chloride	0.1
α-Tocopherol	0.01
Cellulose, Whatman ashless powder	4.0
Bacto-agar, granules	3.0
Sodium alginate (Kelco Company)	0.5
2*M* Potassium hydroxide to pH 6.5	
Water to 100 grams	
Vitamins	*Milligrams*
Niacin	4.0
Calcium pantothenate	2.0
Riboflavin	1.0
Thiamin hydrochloride	0.5
Pyridoxine hydrochloride	0.5
Folic acid	0.5
Biotin	0.02
B_{12}	0.002

Lipides tested.—Oleic, linoleic, and linolenic acids of high purity were obtained from the California Foundation of Biochemical Research. Lard was partially hydrogenated, so that the only unsaturated fatty acid remaining was oleic acid.[5] The methyl ester of linoleic acid and its urea adduct were prepared in our laboratory in the following way.

Refined cottonseed oil (Wesson oil) was treated at room temperature with 1.6 equivalents of anhydrous methanol per equivalent of neutral oil. Sodium hydroxide, 0.5% on the basis of the oil, was used to catalyze the reaction. The reaction mixture was shaken for 2 hours; then the glycerol layer was withdrawn. The ester layer was washed free of glycerol and unreacted methanol. The methyl esters finally were distilled under high vacuum. The purity of the methyl linoleate was checked by an approved method of the American Oil Chemists' Society (1953).

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[2] Entomology Research Division, Agricultural Research Service, U.S.D.A.

[3] Texas Agricultural Experiment Station, College Station.

[4] Taken in part from the thesis of Dundappa Kerur in partial fulfillment of the degree of Master of Science. Mr. Kerur's work was supported in part by a grant from the Robert A. Welch Foundation. His present address, Department of Chemistry, University of Texas, Austin.

[5] Obtained through the courtesy of Robert King, of Mrs. Tucker's Foods, Sherman, Texas.

Table 1.—Weights and emergence of pupae reared on media containing different levels of linoleic acid and linolenic acid.

Grams per 100 Grams of Medium	Weight				Total Number of Pupae	Per Cent Emergence		
	Males		Females					
	No.	Mg.	No.	Mg.		None	Partial	Complete
Linoleic acid								
0.05	9	13.3	20	18.2	29	17	79	3
.075	12	16.0	19	18.0	31	48	0	52
.10	11	15.9	23	19.8	34	12	9	79
.125	23	16.5	38	19.5	61	8	0	92
Linolenic acid								
0.05	12	14.2	13	17.8	27	11	0	89
.075	7	15.4	17	16.7	24	0	0	100
.10	10	16.7	20	18.3	30	7	3	90
.125	19	14.7	9	18.8	31	10	0	90

RESULTS.—*Corn oil.*—The omission of corn oil from the medium resulted in failure of the moths to emerge from their pupal cases. At suboptimal levels (table 3) a few normal moths emerged, but many other moths emerged with their wings still attached to the pupal cases. Furthermore, there was a tendency for the deficient pupae to be smaller (table 2).

Linoleic acid.—The results with linoleic acid were similar to those with corn oil. The effect of four concentrations of linoleic acid on emergence is given in table 1. Nearly normal emergence was obtained with 0.125 gram of linoleic acid per 100 grams of medium. At lower levels the per cent emergence increased with increasing amounts of linoleic acid in the medium. There was very little difference in weight between the pupae of deficient and nondeficient females. There appeared to be more difference between the males, but the samples were smaller. These data show that type of emergence is a better measure of linoleic acid deficiency than pupal weight. The length of the larval period was similar at all levels of linoleic acid tested.

Methyl linoleate and methyl linoleate-urea adduct.—The effects of methyl linoleate were similar to those of linoleic acid, as was expected. One of the difficulties of using unsaturated fatty acids in the medium was that they developed oxidative rancidity rapidly when stored. The preservation of these acids prior to use can be accomplished by preparing their urea adducts, which according to Schlenk & Holman (1950) are stable compounds not oxidized by air. The methyl linoleate-urea adduct contained four times as much urea as linoleate, and the addition of this large amount of urea into the medium introduced the problem of possible urea toxicity.

In table 2 are given the weights of pupae obtained by the use of media with four levels of methyl linoleate-urea adduct and one level of urea plus the optimum amount of corn oil. The inclusion of urea appeared to have no effect upon the size of the pupae up to levels of 1.0 gram of the adduct, equivalent to 0.75 gram of urea per 100 grams of medium. However, at the 2.0-gram level or 1.5 grams of urea, most of the larvae died, only two adults being reared from 30 larvae. These two were small and grew slowly. The rest died in the second instar. Urea appeared to have no effect on emergence of those that reached the pupal stage. Therefore, it was concluded that urea adducts would be a satisfactory substitute for corn oil in the medium.

Linolenic acid.—The results with linolenic acid used at the same levels as linoleic acid are presented in table 1. Emergence was nearly complete for all levels. From these data it was concluded that linolenic acid is more effective than linoleic acid in promoting emergence. Confirmatory evidence of the higher potency of linolenic acid was obtained with linseed oil (table 3). Linseed oil contains 50 to 55% of linolenic acid and 20 to 25% of linoleic acid, or a total essential fatty acid content approximately twice that of corn oil. Yet it required only one-fifth the level of linseed oil as corn oil, 0.05 gram as compared to 0.25 gram per 100 grams of medium, to produce nearly optimum emergence.

Other lipides.—The results of feeding various lipides

Table 2.—Weights of pupae reared on a fat-free medium or on media containing corn oil or methyl linoleate-urea adduct.

Grams of Lipide per 100 Grams of Medium	Approximate Grams of Linoleic Acid	Weight			
		Males		Females	
		Number	Milligrams	Number	Milligrams
None	—	25	13.4	34	15.8
Corn oil					
0.25	0.10	9	18.1	12	20.4
0.25 and urea 0.3	.10	9	15.1	18	19.7
Methyl linoleate-urea adduct					
0.2	.05	17	14.7	41	16.7
0.4	.10	25	16.1	31	18.5
1.0	.25	10	14.6	20	18.2
2.0	.50	—	—	2	11.3

Table 3.—Emergence of moths from pupae reared on media containing various lipides.

Lipide	Grams per 100 Grams of Medium	Number of Pupae	Per Cent Emergence		
			None	Partial	Complete
None	—	87	100	0	0
Corn oil	0.10	23	39	43	18
	.25	63	6	0	94
Oleic acid	.25	10	40	60	0
Hydrogenated lard	.5	39	100	0	0
Vegetable shortening	.5	52	42	58	0
Linoleic acid, rancid	.1	103	13	81	6
	.3	30	10	0	90
Linseed oil	.05	16	12	0	88
	.10	13	0	0	100
	.25	12	0	8	92

are given in table 3. Emergence appeared to increase with the amount of unsaturation. Corn oil at the 0.1-gram level permitted only 18% emergence, whereas 0.25 gram of corn oil per 100 grams of diet gave 94%. No improvement in the per cent emergence was obtained above the 0.25-gram level. Oleic acid was inactive as a replacement for corn oil. Although oleic acid failed to promote emergence, 60% of the moths emerged partially, indicating that it might have a sparing effect on the polyunsaturated fatty acids. With hydrogenated lard containing no polyunsaturated fatty acids all insects died in the pupal stage, but with vegetable shortening 58% emerged partially. The fact that some types of vegetable shortening are made by blending the hydrogenated vegetable oil with the unsaturated oil may explain this emergence.

The development of oxidative rancidity in linoleic acid destroyed its biopotency, as was to be expected. At a level that should produce nearly normal emergence with linoleic acid, 0.1 gram per 100 grams of medium, oxidized linoleic acid permitted only 6% emergence. When the oxidized material was increased to 0.3 gram per 100 grams of medium, 90% of the moths emerged. However, the larval period was 10 days longer than that for insects reared on pure fat or fatty acids. Furthermore, the pupae were smaller, about 11 mg. as compared with a normal of 18 to 20 mg.

Linseed oil, already mentioned as a natural fat high in linolenic acid, was very active even at a 0.05-gram level, which contains approximately 0.025 gram of linolenic acid and 0.01 gram of linoleic acid.

DISCUSSION.—These results with linoleic and linolenic acids are not in agreement with the work of Fraenkel & Blewett (1946), who reported that the two acids are similar in effect. Furthermore, several studies with rats have shown that linolenic acid does not replace linoleic acid in the diet (Deuel & Reiser 1955). The findings herein suggest a different function of these polyunsaturated fatty acids in different animals.

It was noted previously (Vanderzant & Reiser 1956a) that oxidation not only partially destroyed the biological activity of the fat in its effect on emergence, but also produced abnormal markings on the pupae. The pupae also were much smaller, which does not occur with linoleic acid deficiency alone. The toxicity of oxidized products of the essential fatty acids has been observed by other workers. Rats fed with auto-oxidized fatty acids exhibited a retarded growth, decrease in weight, and loss of hair around their mouths and legs, according to Kaneda & Ishii (1954). These workers attributed this to the fatty acid peroxides. Holman & Greenberg (1954) observed no arachidonic acid synthesis when linoleate peroxide was fed to rats.

Many more studies need to be made on the functions of the various polyunsaturated fatty acids. Arachidonic acid, which is apparently the most active essential fatty acid in the rat, has not yet been tested on the pink bollworm.

CONCLUSIONS.—Corn oil, included in the medium for the pink bollworm, supplies this insect with the essential fatty acid, linoleic acid.

Methyl linoleate-urea adduct can be used in place of linoleic acid since urea is nontoxic at low levels in the medium.

Linolenic acid also has essential fatty activity and appears to be more active in promoting emergence of moths than linoleic acid.

The biopotency of fats increases with an increase in the content of polyunsaturated fatty acids in the medium.

Linoleic acid loses most of its essential fatty acid activity upon becoming rancid and it also appears to be toxic.

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Cotton Yield and Quality Losses Caused by Various Levels of Pink Bollworm Infestations[1]

J. R. Brazzel[2] and J. C. Gaines[3]

Pink bollworm (*Pectinophora gossypiella* (Saund.)) infestations of cotton result in lowered yields and quality of the cotton produced. Most of the work to date has been designed to measure reductions in yields caused by this insect pest. Recently research procedures have been employed that allowed evaluation of loss caused by reduction in yield and loss caused by reduction in quality (Anonymous 1954, Brazzel & Gaines 1956, Bishopp 1956).

Most of the loss sustained by pink bollworm damaged cotton resulted from lowered quality until the infestation level became abnormally high, (i.e. 100% of bolls infested with over 10 larvae per boll) (Brazzel & Gaines 1956). The experiments reported herein were designed to obtain additional information on the relative amounts of yield and quality losses caused by lower levels of pink bollworm infestations. This information is needed to determine the insect infestation level at which it becomes economically feasible to begin chemical control practices.

Methods.—The procedure used to obtain the various pink bollworm infestation levels and exclude other cotton pests from the plots was a modification of that described by Brazzel & Gaines (1956). Caged plots 1/200 acre in size were arranged in a randomized block design in 1955 and in a Latin square design in 1956.

Treatments consisted of three levels of pink bollworm infestation and an insect-free check, each replicated four times. Moths were released in each of three treatments at the rate of 15 moths per cage per week over a 3-week period in the 1955 experiment, beginning when the cotton had at least five or six squares per stalk. The same procedure was followed in 1956 except that six adults, three males and three females, were introduced each time. Different levels of infestation were obtained by treating the plots at the rate of 1½ pounds technical DDT per acre at 5-, 7- and 10-day intervals for the light, medium and heavy infestations, respectively. Treatments were started 10 days after the first moth release and continued until harvest. The check plots were treated with Guthion at ¾ pound per acre on the same schedule as the light treatment. Schradan was included in one spray application to control mites and aphids. Insecticides were applied with a 3-gallon Hudson sprayer at the rate of 10 gallons of material per acre.

Infestation records were obtained in the same manner as described by Brazzel & Gaines (1956). The 1955 experiment was defoliated with sodium arsenite on November 5. The experiment was started so late in the year that cool weather delayed maturity and many bolls were not open when the cotton was harvested November 20. The unopened bolls were collected and spread in the greenhouse until open. Then all lint was picked from the burs before ginning since many bolls did not open fully. Cotton in the 1956 experiment was defoliated with aminotriazole August 6 and snapped August 21.

All cotton samples were ginned in the Cotton Section Laboratory of the Texas Agricultural Experiment Station in a power-operated, 20-saw gin equipped for ginning snapped cotton. A sample of lint was sent to the Cotton Ginning Research Laboratory at Mesilla Park, New Mexico for fiber tests. The remainder of the lint was sent to ACCO Fiber and Spinning Laboratory, Houston, Texas for staple, grade, spinning tests and valuation. Seed samples were evaluated by the Cottonseed Products Research Laboratory at Texas A. and M. College.

Losses in value of the cotton produced caused by reduction in quality were obtained by computing the value of the cotton produced in each treatment at the same price paid for the check. The difference of the figure obtained and that actually received for the cotton produced in each treatment was considered loss in quality. Loss in yield was considered the difference between the return for the cotton produced in the untreated check and the value of the cotton produced in each treatment computed at the same price paid for the check.

Results.—The pink bollworm infestation at each bloom and boll count is given in tables 1 and 2 for the 1955 and 1956 experiments, respectively. In the 1955 experiment the infestation was heavy initially in all plots, including the check plots. This experiment was conducted with cut-back cotton which had been heavily infested with pink bollworm. Apparently pupae were in the soil and the adults emerged after the plots were caged resulting in heavy infestations in all plots. However, the insecticide treatments brought the infestation levels down rapidly before many bolls were present as indicated by the September 22 bloom count. The boll infestations nearly doubled over the 4-week period of boll counts with the final count within the desired range, table 1.

In 1956 the plots were caged early enough to prevent any natural infestation and the check plots were practically insect-free throughout the season, table 2. The data for bloom infestations for this experiment show the rapid rate of build-up of which this insect is capable. Three male and three female adults were released in each treatment on June 18. On June 27 no rosette blooms were found but on the following day 1.3 to 3.3% of the blooms were infested and by June 30 the infestation had reached 20% in one treatment. The boll infestations increased slightly each week over a 5-week period and never reached 100% of the bolls infested. Thus, the infestation levels for the two experiments ranged from 60% bolls infested with 2.4 larvae per infested boll and an insect-free check in 1956 to 100% bolls infested with 2.4 larvae per infested boll in 1955.

The tests on seed and results obtained for the two ex-

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Table 1.—Bloom and boll infestations for evaluation studies of pink bollworm damage to cotton during the fall of 1955 at College Station, Texas.

	Per Cent Blooms Infested							
	September				October			
Treatment	14	16	22	29	6	13	20	27
Check	26.9	25.8	0.6	0.4	0	1.6		
Light	28.6	37.1	4.0	5.4	4.9	8.5		
Medium	14.3	35.1	2.5	8.2	7.4	11.2		
Heavy	15.6	42.9	5.7	10.8	17.8	25.8		

	Per Cent Boll Infestation							
	Per Cent	Larvae per Boll	Per Cent	Larvae per Boll	Per Cent	Larvae per Boll	Per Cent	Larvae per Boll
Check	37	1.1	15	1.6	12	0.8	17	1.4
Light	51	1.3	53	1.6	67	1.3	98	1.9
Medium	52	1.2	59	1.4	65	1.4	100	2.4
Heavy	61	1.2	73	1.7	89	2.2	100	2.4

Table 2.—Bloom and boll infestations for evaluation studies of pink bollworm damage to cotton during the spring of 1956 at College Station, Texas.

	Per Cent Blooms Infested									
	June				July					August
Treatment	27	28	29	30	3	5	12	19	26	2
Check	0	0	0	0	0	0	0.5	0		
Light	0	1.3	7.8	18.5	16.5	24.5	6.0	0.5		
Medium	0	3.3	6.5	20.0	22.3	25.5	9.8	1.3		
Heavy	0	3.3	6.3	13.3	19.8	25.5	8.5	1.8		

	Per Cent Boll Infestation									
	Per Cent	Larvae per Boll	Per Cent	Larvae per Boll	Per Cent	Larvae per Boll	Per Cent	Larvae per Boll	Per Cent	Larvae per Boll
Check	2	0	2	1.0	0	0	0	0	0	0
Light	48	1.1	43	1.1	40	1.3	48	1.8	60	2.4
Medium	51	0.9	49	1.1	43	1.2	81	1.8	86	1.8
Heavy	39	1.3	44	1.0	41	1.3	74	1.6	86	2.3

periments are shown in table 3. In the 1955 experiment there was a significant increase in dirt and trash in the treatments. All other differences were not significant. In 1956 there was a significant increase in free fatty acids and lint, and significant decrease in oil, ammonia and protein. Differences in dirt and trash and moisture were not significant. These data for both experiments show that relatively slight increases in insect infestation cause lowered quality of the seed.

The yields, results of lint tests, and value per acre are given in table 4 for both experiments. Yields per acre of both lint and seed decreased slightly with increased levels of insect infestation in both experiments. Even though the differences were not significant, a trend of decreased

Table 3.—Cotton seed tests and grade of seed produced at various levels of pink bollworm infestation at College Station, Texas. 1955–56.

		Per Cent							Index		
Per Cent Infestation	Larvae per Boll	Dirt and Trash	Moisture	Free Fatty Acids	Oil	Ammonia	Protein	Lint	Quantity	Quality	Grade
					Fall of 1955[a]						
17	1.4	0.6	9.3	0.4	15.3	3.9	20.0	13.5	86.6	100.0	86.5
98	1.9	1.1	9.7	0.5	14.8	3.7	19.4	12.9	83.2	99.8	83.0
100	2.4	1.0	10.1	0.4	15.5	3.6	18.7	13.1	86.4	99.9	86.3
100	2.4	1.3	10.7	0.5	15.0	3.7	18.9	14.1	83.9	99.6	83.6
L.S.D. at 5% level	0.4										
					Spring of 1956						
0	0	0.8	6.6	0.3	16.4	4.7	24.3	9.0	98.5	99.9	98.4
60	2.4	1.0	6.7	0.6	15.4	4.6	23.6	9.7	91.8	100.0	91.8
86	1.8	1.8	6.9	1.9	13.4	4.2	21.6	11.4	77.5	98.8	76.6
86	2.3	1.3	7.1	1.6	13.9	4.5	22.9	10.3	81.9	99.4	81.4
L.S.D. at 5% level				0.6	1.7	0.2	1.4	1.2			

[a] Cotton handpicked.

Table 4.—Yield, lint tests, and value per acre of cotton produced at various levels of pink bollworm infestation at College Station, Texas. 1955–56.

Per Cent Infestation	Larvae per Boll	Yield/Acre		Fiber			Uniformity Index	Staple	Grade	Value ($/A.)
		Lint	Seed	Strength	Maturity	Fineness				
						Fall of 1955[a]				
17	1.4	530	889	107	62	3.0	76	15/16 to 1	GO to SGO	121.45
98	1.9	508	824	105	64	3.1	77	15/16 to 1	GOEW to SGOEW	122.43
100	2.4	472	764	105	68	3.1	78	29/32 to 31/32	OEW to SGOEW	112.64
100	2.4	433	750	106	66	3.1	77	15/16 to 31/32	O to GOEW	100.09
						Spring of 1956				
0	0	481	857	108	73	4.1	81	15/16 to 31/32	GOEW to SGOEW	130.50
60	2.4	469	813	107	72	4.1	82	29/32 to 31/32	GOEW to SGOEW	120.39
86	1.8	443	700	108	69	3.8	80	7/8 to 1	OEW to GOEW	98.20
86	2.3	465	757	107	71	3.9	80	15/16	OEW to GOEW	107.73

[a] Cotton handpicked.

yields with increased pink bollworm infestations was indicated.

Results of the fiber tests indicated no significant differences, nor do they follow the same trend mentioned above for yields. Higher values were obtained in all tests for the 1956 experiment than for 1955. This apparently resulted from the fact that in 1955 cold weather late in the season delayed maturity of the cotton so much that it was necessary to harvest the experiment before all bolls were open.

Staple and grade were practically the same for the light level of insect infestation and the check in both experiments, ranging from good ordinary to strict good ordinary extra white. Only at the intermediate and heavy levels of infestation were grades obtained below good ordinary. In both experiments the range of grades of cotton from plots within each infestation level overlap grades of cotton from plots of all other infestation levels. Staple varied only slightly throughout the range of infestations of both experiments. The same overlapping of staple length occurred in both experiments as described for grade.

The value of lint and seed produced was greater in the light level of insect infestation than in the check in the 1955 experiment. In all other treatments the value for cotton produced was about 10 dollars per acre less between treatments as the insect infestation increased, table 4.

Figures 1 and 2 illustrate graphically the returns in dollars per acre for lint and seed, respectively. Returns for the cotton produced in the experiment reported by Brazzel & Gaines (1956) are plotted in these graphs as spring 1955, for the purpose of comparing the data reported herein with extremely high levels of pink bollworm

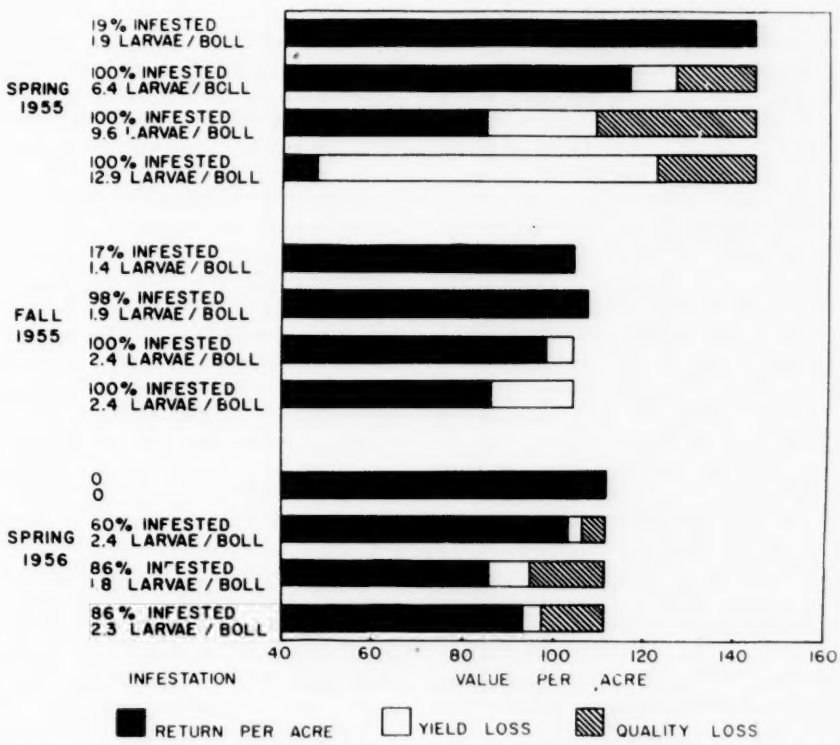

FIG. 1.—Returns for lint in dollars per acre for cotton produced in three experiments with various levels of pink bollworm infestation, and evaluation of losses caused by reduced yield and quality.

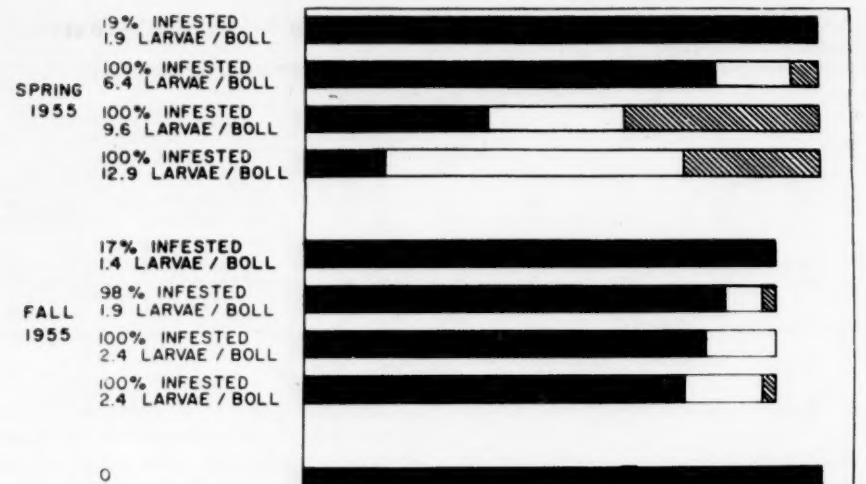

FIG. 2.—Returns for cottonseed in dollars per acre for cotton produced in three experiments with various levels of pink bollworm infestation, and evaluation of losses caused by reduced yield and quality.

infestation. The loss in value of the lint and seed in each treatment caused by reduced yield and reduced quality are shown by these figures.

The most obvious feature of the data presented in figure 1 is that no loss caused by reduced quality occurred in the experiment conducted in the fall of 1955. Also, the return was higher in the light level of insect infestation in which 98% of the bolls were infested and contained an average of 1.9 larvae per infested boll. Reduced returns that were caused by reduced yields were evident in the intermediate and heavy infestations. The infestations given in the graphs were those existing at the final boll examination. Using this single record is slightly misleading as illustrated in the results of the experiment conducted in the fall of 1955. The insect infestation was identical for the last two treatments, yet the yield loss was almost three times as great in the last treatment as in the other one. Examination of table 1 shows that the pink bollworm infestation was greater in all counts in the last treatment except for the final count.

Data presented in figure 1 for experiments conducted in the spring of 1955 and 1956 indicate that most of the losses were caused by reduced quality of the lint until the insect infestation reached abnormally high levels of 100% bolls infested and over 10 larvae per boll. In the experiment conducted during the spring of 1956 the loss caused by reduced quality was almost two times as great as that caused by reduced yields. The reasons for lack of quality losses in the fall experiment of 1955 is not known. It seems unlikely that handpicking the cotton could have been a factor since a conscientious effort was made to pick all lint even though it was damaged. It seems more likely that quality losses were obscured by the immaturity of the cotton which is shown in the data in table 4.

The loss in value caused by reduced yield and quality of seed is shown in figure 2. In most cases the loss resulting from lowered yields equals or is greater than that for lowered quality of the seed. These results indicate that a greater percentage of the loss in value of lint is caused by reduced quality than in the case of the seed.

SUMMARY AND CONCLUSIONS.—Experiments were conducted, in the fall of 1955 and in the spring of 1956, to determine the effect on the yield and quality of cotton having moderate levels of pink bollworm infestations approaching those that are usually found in infested fields. Approximately two-thirds of the loss in value of lint was caused by reduced quality when the cotton was snapped. Lint-yield losses occurred but no quality losses were evident for handpicked cotton. Loss in value of seed was about equally divided between reduced yields and quality.

These results indicate that pink bollworm infestation levels up to about 50% infested bolls and less than 2 larvae per infested boll cause relatively small decreases in value of the cotton produced. In one experiment, in which the cotton was handpicked, the value per acre was greater for the light infestation level than for the uninfested check. In the other experiment, in which the cotton was snapped, the value of the light infestation level was reduced about 10 dollars per acre compared with the

check, yielding about 1 bale per acre. Although the yield differences for seed and lint were not significant, there was a trend towards lowered yields with increase in infestation.

The two experiments reported herein were conducted under very dry weather conditions. Wet weather during the month preceding harvest, when pink bollworm emergence holes were present in bolls, might have caused further reduction of yields and quality by boll rots and staining of lint.

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Control of House Fly Larvae in Poultry Houses[1]

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The control of house flies (*Musca domestica* L.) by means of larvicides received only casual attention for several years, while residual sprays against adults were giving highly satisfactory results. The report by Bruce & Decker (1950) that the development of resistance is accelerated when both adults and larvae are treated with insecticides also discouraged the use of larvicides. Since adequate house fly control has become increasingly difficult, especially around poultry and livestock establishments, there has been considerable interest in the use of organic-phosphorus compounds as larvicides.

In laboratory tests Kocher *et al.* (1953) showed that Diazinon was effective at 5 p.p.m. against full-grown larvae that had become resistant to chlorinated hydrocarbon insecticides. Mayeux (1954 *a* and *b*) reported that sprays and granules containing 1% of malathion were very effective against fly larvae in manure. The sprays were applied at 1 gallon and the granules at 1.5 pounds per 100 square feet. Sampson (1956) found Diazinon superior to malathion and nearly as effective as parathion when infested organic matter was treated with liquid sprays. Hoffman & Monroe (1956) evaluated five phosphorus compounds in poultry droppings in California. They obtained 100% mortality in 24 hours with Diazinon and Dipterex at 100 mg. or more per square foot, but dosages as high as 1000 to 1500 mg. were needed to give 93–95% control with malathion and Am. Cyanamid 4124. Chlorthion was the least effective. Lindquist & Fay (1956) in laboratory tests determined the order of decreasing effectiveness to be Diazinon, EPN, parathion, Bayer 21/199, NPD (tetrapropyl dithiopyrophosphate), demeton, Dipterex, and malathion.

In many parts of the country it is customary to keep the laying flock, young chickens, and broilers in rows of wire cages about 3 feet above the ground. The manure is allowed to accumulate under the cages, where it forms a cone that is sometimes 2 or 3 feet in height. When the manure remains dry, fly densities are low. However, when the manure becomes moist or wet from drifting rains, spilled water or diarrheal chickens, house fly and soldier fly (*Hermetia illucens* L.) larvae quickly get a start. Unless corrective measures are taken immediately, the entire pile soon becomes infested and a major fly problem results.

TESTS WITH LIQUIDS.—Eight organic-phosphorus compounds were tested as larvicides against natural populations of house flies breeding in manure under caged poultry in the vicinity of Orlando, Fla. Water solutions of Dipterex and emulsions of the other insecticides were applied with a garden sprinkling can. Various concentrations were used at 50 to 100 ml. of liquid per square foot of surface. Larval density was evaluated by collecting a spoonful of manure from enough different sites in each plot to make a 1-pint sample, and counting the larvae after washing the sample through a screen. Results of these tests are shown in table 1.

Table 1.—Control of house fly larvae in poultry manure with organic-phosphorus compounds applied in emulsions or solutions.

LARVICIDE	MILLIGRAMS PER SQUARE FOOT	PRETREATMENT COUNT	PER CENT CONTROL AFTER— 2 DAYS	1 WEEK	2 WEEKS
Diazinon[a]	200	310	98	100	58
	100	300	84	100	96
Dipterex	250	568	93	0	—
	100	1230	99	100	5
		99+	100	5	—
Bayer 21/199	300	2180	100	0	—
	200	1832	100	0	—
	100	1756	100	0	—
Dimethyl trichlorophenyl phosphorothioate	100	620	72	100	6
Am. Cyanamid 4124	200	725	97	77	0
Malathion	250	1433	92	15	—
	200	1825	95	77	0
	100	2263	96	73	0
		224	0	0	—
Chlorthion	200	1226	96	9	—
		1110	0	—	—
Parathion	200	1645	0	—	—
	100	1240	0	—	—

[a] No control after 3 weeks with either dosage.

Diazinon was effective 1 to 2 weeks, Dipterex and Dow ET-57 (sampled as Dow ET-14) for 2 days to 1 week, Bayer 21/199 and Am. Cyanamid 4124 for 2 days, malathion and Chlorthion for 2 days in some tests but

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not in others, and parathion was ineffective. Treatments of 100 mg. per square foot were as effective as heavier dosages. However, the water added with these treatments increased the fluidity of the manure, causing it to become unsalable and extremely difficult to handle.

TESTS WITH DUSTS.—Tests were conducted to determine whether applying Diazinon in a dry form would prevent liquefaction of the manure as well as larval development. The 25% Diazinon consisted of 1.86 pounds of wettable powder added to 36 pounds of attapulgite and 2 pounds of celite. It was sprinkled from a 1-pint flour scoop onto the manure at 150 mg. of Diazinon per square foot.

Tests were begun on a chicken farm where most of the manure was liquid and there were large numbers of both soldier fly and house fly larvae distributed in all areas. Two days after the initial dust application was made on June 13, 1956, only one soldier fly and no house fly larvae were found. On June 20 the manure was still liquid; a few house fly larvae were observed and a second application was made. Two days later only one soldier fly larva could be found, but on July 5 a few house fly larvae were present. The manure was removed about July 9; it was still soft, but much improved since the beginning of the test.

On July 13 weekly treatments were begun on freshly stacked manure on the same farm. Numerous house fly larvae were found in pockets before treatment, but all were dead after 48 hours. From July 18 through September very few larvae were found at any time except during the week of September 5. Laboratory tests showed the wettable powder used for this treatment was defective, and the application made on September 12 with a different powder gave almost complete control. At times the manure was slightly liquid in spots, caused by the liquid nature of the droppings of a few chickens, but in general it remained dry.

On November 7 another poultryman with approximately 1,500 chickens reported that fly larvae were rapidly liquefying the manure beneath his cages and his efforts at control had failed. A pretreatment inspection showed the manure to be heavily infested with all stages of house fly and soldier fly larvae. A single application of the Diazinon powder reduced the infestation to such a degree that within 24 hours only isolated pockets of both species of larvae remained and the control was estimated to be approximately 95%. The following day the control reached 99%, and 1 and 2 weeks later no larvae could be found. During the third week a few house fly and soldier fly larvae were present, and by the fourth week reinfestation had occurred in all areas.

The dust appears to have a distinct advantage over the liquids. Instead of further liquefying the medium, it helps dry it out and thereby makes it less favorable for breeding. Also uniform coverage can be assured, as the dust is readily visible. If this treatment is begun early in the season, it should be possible to maintain adequate control.

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Further Field Tests with Systemic Insecticides Employed as Seed Treatments[1]

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Field experiments conducted near Waco, Tex., in 1954 and 1955 with Am. Cyanamid 12009, 12008, and Thimet (Am. Cyanamid 3911) employed as seed treatments against thrips (*Frankliniella* sp.), the cotton aphid (*Aphis gossypii* Glov.), the serpentine leaf miner (*Liriomyza pusilla* (Meig.)), the cotton fleahopper (*Psallus seriatus* (Reut.)), and the overwintered boll weevil (*Anthonomus grandis* Boh.) have been reported (Parencia *et al.* 1957). It is the purpose of this paper to discuss the comparative effectiveness of a new systemic compound, Bayer 19639, and Thimet against various early-season cotton insects in field experiments conducted in 1956. A brief report of grower experience with Thimet is included.

MATERIALS AND METHODS.—Both Bayer 19639 and Thimet were obtained from the manufacturer as 50% activated-charcoal dusts. Planting seed was treated in a mechanically rotated drum about 1 month before planting. Mechanically delinted (reginned) seed that was to carry 1 pound of toxicant per acre was treated at the rate of 6 pounds of the dust per 100 pounds when the planting rate was to be 33 pounds per acre, and at 8 pounds when the planting rate was to be 25 pounds. Acid-delinted seed was treated by a local seed-treating company at the rate of 8 pounds of the dust per 100 pounds. In the tests in which 2 pounds of 19639 was to be used, a small amount of a Vatsol-Methylcel mixture was added as a sticker in

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treating reginned seed. Granulated formulations contained 2% of the insecticides.

Two- or four-row equipment available on the various farms was used to plant the seed. In two experiments 2% of granulated Thimet was applied at 52.7 or 64.7 pounds per acre with a fertilizer distributor in a band about 4 inches below the drill in which treated seed carrying a 1-pound dosage was planted. This was done in an effort to determine if additional Thimet made available in this way would extend the effective period of the seed treatment. In one experiment 2% of granulated Bayer 19639 at 55 pounds per acre was applied slightly above the plants with a combination seed and fertilizer spreader. Distributor holes were plugged so that the insecticide was not broadcast but was applied over the drill of the rows. A conventional spray treatment (toxaphene applied at weekly intervals for 4 to 5 weeks) was included in three of four experiments.

One experiment was conducted in small plots and three in large plots. The small plots were 24 rows wide and of sufficient length for an area of 0.2 acre. The plots were arranged in randomized blocks with five replications of each treatment. The large plots ranged from 1 to 6 acres in size, and there were no replications of treatments.

EFFECT ON PLANT EMERGENCE.—In experiments 1, 2, and 3 the seed was planted in mid-April under conditions of dry, cool weather. To insure adequate moisture, locations were selected in fields in which the seed beds had not been reworked during the winter and spring. High, dry winds made it necessary to plant and cover the seed much more deeply than normally. Stands were obtained in 2 weeks, and there were no significant differences between those obtained from treated and untreated seed in the small-plot experiment and no appreciable differences in the large-plot experiments (table 1).

The field in which experiment 4 was to be located was flooded during the May 1 rains by an overflow of Tehuacana Creek. The seed was planted over a 3-day period, May 14 to 16; so differences in plant emergence were difficult to evaluate. However, emergence was reduced and delayed in the plot in which granulated Thimet was placed below the drill in which Thimet-treated seed was planted. The soil was wet at the time of planting, and the deeper opening made to place the granules permitted a greater degree of drying than in the normally planted plots. This condition rather than the seed treatment affected the stand, although it was considered to be adequate.

Cotyledons of plants grown from treated seed showed some phytotoxic effects in all experiments, but injury was not severe. Phytotoxicity appeared to be somewhat less from 1 pound of Bayer 19639 than from 1 pound of Thimet, although in experiment 3 the difference was slight. At 2 pounds it was about the same or slightly greater than at 1 pound of Thimet. Phytotoxicity to true leaves occurred only in the plots in which granulated Thimet was placed below treated seed, and then only in small areas in which the granules were placed too near the seed owing to variation in equipment adjustment. Where placement was made at the 4-inch depth this condition did not occur.

EFFECTIVENESS AGAINST THRIPS.—Results against thrips are shown in table 2. In experiment 1, 3 weeks after plant emergence there were no significant differences in control between treated-seed and toxaphene-spray plots, but all of them had significantly lower infestations than the check. After 4 and 5 weeks toxaphene spray gave significantly better control than either of the seed treatments, which were no better than the check. When the seasonal average was considered, the seed treatments and toxaphene spray reduced the infestation significantly below that of the check, and toxaphene spray was better than Bayer 19639.

In experiment 2, 3 weeks after plant emergence Thimet applied to reginned seed gave better control than when it was applied to acid-delinted seed or than when Bayer 19639 was applied to reginned seed. The superiority of the reginned-seed treatment was apparently due to better adherence of the toxicant to the seed. There was a tendency for the material to flake off from the acid-delinted seeds, and a greater amount of the mixture remained in the bags containing them after they were poured into the planter hoppers and in the hoppers after planting was completed. All seed treatments showed loss of effectiveness after 4 weeks.

In experiment 3 the infestation was not as heavy as in the preceding experiments. Both Thimet seed treatments gave better control 3 and 4 weeks after plant emergence

Table 1.—Planting and plant-emergence records in field experiments with Thimet and Bayer 19639 employed as cottonseed treatments.

INSECTICIDE (1 POUND PER ACRE UNLESS OTHERWISE INDICATED)	POUNDS OF SEED PER ACRE	NUMBER OF SEEDLINGS PER 100 FEET OF ROW	
Experiment 1 (Planted April 17)			
		April 30	*May 14*
Thimet	32.3	496	548
Bayer 19639	30.2	446	472
Toxaphene spray	36.2	470	508
Untreated check	36.2	534	514
Experiment 2 (Planted April 16–17)			
Thimet	33.7	557	558
Thimet[a]	25.8	441	467
Bayer 19639	35.2	494	522
Untreated Check	31.1	552	545
Experiment 3 (Planted April 17–18)			
		May 7	*May 22*
Thimet	24.1	347	364
Thimet+granules	25.4	385	334
Bayer 19639			
1 pound	25.1	334	314
2 pounds	26.2	350	327
Toxaphene spray	25.0	368	371
Untreated check	25.5	389	381
Experiment 4 (Planted May 14–16)			
		May 23	*June 6*
Thimet	33.5	249	310
Thimet+granules	31.8	169	287
Thimet[a]	30.1	496	461
Bayer 19639			
1 pound	32.3	317	446
2 pounds	35.0	326	333
Toxaphene spray	31.8	484	348
Untreated check	31.8	653	389

[a] Acid-delinted seed.

Table 2.—Effectiveness of Thimet and Bayer 19639 as cottonseed treatments against thrips.

Insecticide (1 Pound per Acre Unless Otherwise Indicated)	Average Number of Thrips per Plant 1 to 5 Weeks After Emergence					
	1	2	3	4	5	Average
Experiment 1						
Thimet	0.1	0.02	1.1	5.4	9.8	3.2
Bayer 19639	.2	.2	1.7	7.4	12.5	4.4
Toxaphene spray	1.6	.4	0.8	2.2	4.7	1.9
Untreated check	1.6	13.9	3.1	6.7	12.3	7.5
L.S.D. at 5% level	.7	4.1	0.9	2.3	4.9	1.3
Experiment 2						
Thimet	0	0.1	0.8	10.9	12.6	4.9
Thimet[a]	0.02	.1	3.0	5.7	12.0	4.2
Bayer 19639	.2	.8	3.3	10.4	10.1	5.0
Untreated check	2.7	17.4	7.2	7.8	10.6	9.1
Experiment 3						
Thimet	0.02	0.05	0.4	1.5	4.8	1.4
Thimet+granules	0	0	.4	1.2	6.2	1.6
Bayer 19639						
1 pound	.1	0	.8	4.4	6.4	2.3
2 pounds	.02	0	1.0	5.0	8.2	2.8
Toxaphene spray						
0.75 pound	.4	.3	0.8	2.8	3.8	1.6
Untreated check	.6	1.1	2.2	4.0	8.2	3.2
Experiment 4						
Thimet	0.02	0.1	1.2	4.7	21.4	5.5
Thimet+granules	0	.2	1.0	4.9	23.4	5.9
Thimet[a]	.02	.9	2.8	8.6	15.6	5.6
Bayer 19639						
1 pound	.02	.2	2.8	6.4	17.4	5.4
2 pounds	0	.2	1.0	3.0	20.0	4.8
Bayer 19639+granules	.02	.2	2.8	5.5	13.9	4.5
Toxaphene spray	.05	2.4	3.2	4.0	6.0	3.1
Untreated check	.3	3.0	5.8	14.7	21.4	9.0

[a] Acid-delinted seed.

than the 1- and 2-pound dosages of Bayer 19639. After 5 weeks toxaphene spray gave the best control, and the Thimet seed treatment was better than the other seed treatments. The placement of granulated Thimet below the drill did not improve control over that afforded by the Thimet seed treatment alone.

In experiment 4, 3 weeks after plant emergence both Thimet reginned-seed treatments and Bayer 19639 at 2 pounds per acre gave better control than the other treatments. After 4 weeks 19639 at this dosage gave the best control, followed by the two reginned Thimet seed treatments and toxaphene spray. After 5 weeks only toxaphene spray gave appreciable thrips control. As in experiment 3, the placement of Thimet granules below the drill did not improve control over the seed treatment alone. Two per cent of granulated Bayer 19639 at 55 pounds per acre applied 3 weeks after emergence over plants grown from seed treated with a 1-pound dosage of 19639 did not materially reduce the infestation.

EFFECTIVENESS AGAINST OTHER EARLY-SEASON PESTS.—Infestations of the cotton aphid and the desert spider mite (*Tetranychus desertorum* Banks) were too low for an evaluation of the seed treatments in their control (table 3). However, in experiment 3 aphid infestations were lower in the treated-seed and check plots than in the plot receiving toxaphene spray.

Cotton fleahopper infestations were low in all experiments, although somewhat higher in experiment 3. The infestation was only slightly lower in the treated-seed plots than in the check, but it was considerably lower in the plot receiving toxaphene spray than in all other plots. When adult fleahoppers were installed on caged plants 2 weeks after their emergence from seed treated with the 1-pound dosage of either insecticide, the net kill was 100%. After 3 weeks the kill in the Bayer 19639 treatment was 78.5%. The cage in the Thimet-treated seed plot was lost; therefore no data were obtained for that week in this plot. After 4 weeks no kill was obtained in either plot.

Boll weevil infestations were rather low in all experiments, although somewhat higher in experiment 3. There was very little difference between the treated-seed plots and the check, but the infestation was considerably lower in the plot receiving toxaphene spray.

Rains occurred on May 1, and for several weeks provided conditions unfavorable for the development of leaf miner infestations. In experiment 3 light infestations

Table 3.—Effectiveness of Thimet and Bayer 19639 as cotton seed treatments against early-season insects other than thrips.

Insecticide (1 Pound per Acre Unless Otherwise Indicated)	Average Number per Plant[a]		Cotton Fleahoppers per 100 Terminals[b]	Weevil-Punctured Squares[c] (Per Cent)
	Cotton Aphids	Spider Mites		
Experiment 1				
Thimet	0.002	0	9.4	—
Bayer 19639	.01	0.002	8.6	—
Toxaphene spray	.19	.027	6.4	—
Untreated check	.22	.005	10.2	—
L.S.D. (5%)	.17	—	—	—
Experiment 2				
Thimet	0	0.007	6.7	—
Thimet[d]	0	0	10.4	—
Bayer 19639	.01	0	9.4	—
Untreated check	.08	0	10.5	—
Experiment 3				
Thimet	0.04	0	14.1	18.2
Thimet+granules	0	0	11.4	16.2
Bayer 19639				
1 pound	.01	0	8.4	13.0
2 pounds	0	0	7.4	20.6
Toxaphene spray				
0.75 pound	1.72	0	4.3	9.0
Untreated check	.04	0	20.0	20.3
Experiment 4				
Thimet	0.01	0	5.5	3.6
Thimet+granules	.04	0	5.1	4.8
Thimet[d]	.11	0	3.1	5.7
Bayer 19639				
1 pound	.11	0	5.4	5.4
2 pounds	0	0	5.7	6.2
Bayer 19639+granules	0	0	4.9	6.9
Toxaphene spray	.16	0	2.5	5.1
Untreated check	.28	0	6.0	5.1

[a] Average of 5 weekly records after plant emergence.
[b] Average of 5 weekly records in experiments 1 and 2 and of 7 weekly records after plant emergence in experiments 3 and 4.
[c] Average of records made 6 and 7 weeks after plant emergence.
[d] Acid-delinted seed.

occurred on all plants in the treated-seed plots and check 5 weeks after plant emergence, an indication that the seed treatments were no longer effective against this pest.

In experiment 1 there were significant increases in plant heights (three biweekly records between May 21 and June 18) in the treated-seed and toxaphene spray plots over the untreated check (not shown in tables). Plants were taller also in the same plots in the large-plot experiments, and differences in plant appearance reflected early thrips control. A count made on June 11 in experiment 1 showed significantly more fruiting forms per plant in the toxaphene-spray than in the treated-seed or check plot. Earliness in fruiting was in favor of toxaphene spray in the other experiments. The seed treatments lost effectiveness against thrips in the early fruiting period (4 or 5 weeks after plant emergence), with consequent delay in fruiting. This was also reflected in bloom records made during the early blooming period. However, there was no evidence of a delay in fruiting beyond that in untreated-seed plots.

There was no appreciable late-season insect damage except in experiment 3. In experiment 1 endrin spray was applied at 0.4 pound per acre at weekly intervals throughout the fruiting period beginning 5 weeks after plant emergence. In experiment 4 three spray applications of toxaphene-DDT for bollworm control were made between July 13 and 23. The field in which experiment 3 was conducted was irrigated in early July, and was then used for a late-season boll weevil control experiment, since locations for conducting such experiments were very scarce and the entire acreage required seasonal protection. The identity of the plots was maintained, and good yields were produced in all the original treated-seed plots. The severe drought seriously reduced yields in experiments 1, 2, and 4 (table 4). Rainfall from January 1 to August 31 was 9.79 inches. Increases in yield, though slight, were in favor of toxaphene spray.

GROWER EXPERIENCE IN THE USE OF THIMET-TREATED SEED.—An estimated 15 to 20 thousand acres of cotton were planted with Thimet-treated seed in Texas in 1956. The seed was treated by two Waco companies, and the cost to the grower was $3 per acre. One pound of Thimet was applied to 25 pounds of cottonseed, which was the planting rate per acre. Questionnaires pertaining to stand and insect control obtained from the seed treatment were mailed to growers who had planted treated seed within 125 miles of Waco. Replies relating to stands were received from 171 farmers involving 6,162 acres, and relating to thrips control from 112. Stands obtained on the various farms were rated as follows: good 55%, fair 28%, poor 11%, and no stand 6%.

Estimated thrips control obtained ranged from 0 to 8 weeks, with a high percentage of the estimated 4, 5, and 6 weeks. The overall average was 4.6 weeks. Of the 112 growers, 94 reported that they were satisfied with the insect control obtained, and 81 indicated that they would plant treated seed again next year. A few others commented that they might use it again if the cost of treatment was cheaper and if weather conditions were more favorable.

Table 4.—Late-season boll weevil, bollworm infestation, and yield records made in experiments in which Thimet and Bayer 19639 were used as cotton seed treatments.

Insecticide (1 pound per acre unless otherwise indicated)	Seasonal average infestation (per cent)			Pounds of seed cotton per acre	
	Boll weevil	Bollworm injury			
		Squares	Bolls	Total	Gain over check
Experiment 1					
Thimet	3.1	3.8	2.7	609	−34
Bayer 19639	3.6	4.1	2.8	641	− 2
Toxaphene spray	3.2	4.0	2.4	699	56
Untreated check	2.9	3.9	3.0	643	—
L.S.D. (5%)	—	—	—	58	—
Experiment 2					
Thimet	8.5	1.4	2.4	433	−13
Thimet[a]	6.7	2.4	6.8	485	39
Bayer 19639	10.0	2.1	3.3	437	− 9
Untreated check	7.5	2.8	3.4	446	—
Experiment 4					
Thimet	6.6	4.3	2.8	370	20
Thimet+granules	7.8	4.8	2.5	400	50
Thimet[a]	6.5	3.0	2.4	399	49
Bayer 19639					
1 pound	8.0	3.6	2.0	373	23
2 pounds	6.9	3.8	2.2	363	13
Bayer 19639+Granules	7.1	2.5	2.5	347	− 3
Toxaphene spray	7.6	4.8	3.2	409	59
Untreated check	12.4	3.9	3.5	350	—

[a] Acid-delinted seed.

SUMMARY.—The effectiveness of the systemic insecticides Thimet and Bayer 19639 as seed treatments was evaluated against thrips (*Frankliniella* sp.) in field experiments conducted near Waco, Tex., in 1956. Some information was also obtained on the control of the cotton aphid (*Aphis gossypii* Glov.), the serpentine leaf miner (*Liriomyza pusilla* (Meig.)), the cotton fleahopper (*Psallus seriatus* (Reut.)), and the overwintered boll weevil (*Anthonomus grandis* Boh.).

Stands were obtained in 2 weeks from plantings made in April when the weather was dry and cool, and in 1 week from those made in May under better moisture conditions. There were no appreciable differences between treated and untreated seed.

In large-plot experiments at 1 pound per acre Thimet was effective against thrips 1 week longer than Bayer 19639. In one experiment Bayer 19639 at 2 pounds per acre was more effective 4 weeks after plant emergence than Thimet at 1 pound. A 1-pound dosage of Thimet applied to reginned seed was effective 1 week longer than when applied to acid-delinted seed. Toxaphene spray applied at weekly intervals was more effective than the seed treatments 4 and 5 weeks after plant emergence.

In the control of cotton fleahoppers and overwintered boll weevils toxaphene spray was more effective than the seed treatments.

Growers who planted Thimet-treated seed were satisfied with thrips control afforded by the treatment.

REFERENCE CITED

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Lice Control on Northern Range Herds with Residual Sprays[1]

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Most of the published work on lice control with residual sprays or dips on cattle has been done in the South and on the west coast (Furman 1947, Kemper *et al.* 1948, Simms 1946, 1949, Smith & Richards 1955, and others), where the season of severe infestation is relatively short, or in the East (Lancaster 1951), where the results were obtained on small dairy herds that could be very thoroughly treated. Good control or even "eradication" was obtained with various chlorinated insecticides. In some of the cases where complete control or eradication was achieved, only a few test animals were involved or the period of observation extended for only a few weeks after treatment.

Observations on northern range herds during the past 5 years would seem to indicate that complete seasonal control is not easily achieved on large herds where the winters are severe. During this period, the effectiveness of single applications of chlordane, heptachlor, lindane, dieldrin, Perthane and malathion for cattle lice control was investigated. These insecticides were evaluated under field conditions on large beef herds in a region where the climate is conducive to heavy infestation for 7 or 8 months of the year.

The work was carried out on the following general lines: (1) 1951–52, determine effectiveness of sprays when applied late after herds are heavily infested. New animals are frequently added to herds or the herds brought in for the winter from outlying pastures late in the fall. For that reason, many ranchers do not begin control operations until December or January after the onset of cold weather; (2) 1952–53, determine whether it is possible to eradicate lice or at least obtain seasonal control by spraying early in the fall, *i.e.*, late September or October, when lice populations are still quite low; (3) 1953–54, determine any advantages of adding BHC or lindane because of possible ovicidal qualities to insecticides of longer residual action; (4) 1954–55, determine effectiveness of malathion; and (5) 1955–56, determine effectiveness of adding malathion as an ovicide to insecticides of longer residual action.

Lice Species.—*Solenopotes capillatus* Enderlein was the major species encountered in this work, which was carried on in southeastern Wyoming. Of 39 tests on 21 herds, *S. capillatus* was detected in 31 tests and represented by far the greatest number of lice. *Linognathus vituli* (L.) was second in importance and was encountered in 34 tests. *Haematopinus eurysternus* (Nitz.) was encountered in 14 tests and *Damalinia bovis* (L.) twice, and on only one animal on both occasions. On the larger range herds, *H. eurysternus* is probably of more importance than indicated here. Snipes (1948) and Denning (1950, unpublished data) found *H. eurysternus* to be the most important species on Wyoming beef cattle.

Materials and Methods.—Only wettable-powder formulations of the various insecticides were used. A wetting agent, Triton B-1956, was added at the rate of 1 pint per 100 gallons of water in the 1953–54 and 1954–55 tests. The applications were made at a pressure of 200 to 400 p.s.i. with a single-nozzle pistol-grip gun having a No. 6, 8, or 10 orifice. The cattle were run into a squeeze pen and sprayed in groups of 6 to 10 at a time. The amount of spray solution per animal varied from much less than 2 gallons for calves sprayed early in the fall to more than 3 gallons for cows sprayed in midwinter when the hair was long. All test herds except one were Herefords.

Treated herds were examined immediately before spraying to determine the degree of infestation and within 1 or 2 weeks after spraying to determine the initial kill. The head, neck, and brisket were the parts of the body thoroughly examined for lice. Very rarely were any lice found on the five to ten animals examined in the first post-spray check. Subsequent examinations were made at approximately 2-month intervals to March or April, although this schedule varied from year to year and was influenced by weather and availability of the herds. Three herds received a second treatment at an interval longer than the recommended 16 to 18 days to determine effectiveness of two treatments when the second treatment must be postponed because of weather. In table 1, the tests are grouped under the year in which conducted, since it is difficult to compare tests conducted in different years. Only the last examination of the herd before lice were found, and subsequent examinations to the end of control, or to the final examination of the season, are shown.

Ovicide tests were conducted in 1953 with the insecticides listed in table 2, except malathion, which was tested in 1955. Hereford cows heavily infested with *H. eurysternus* were sprayed individually with 3 to 4 gallons of water suspension sprays made from wettable powders to which Triton B-1956 at the rate of 1 pint per 100 gallons of water had been added. Samples containing 100 to 400 eggs of lice were collected immediately before spraying, 1 hour and 48 hours after spraying, held overnight at room temperature, and then incubated for 14 days at 32° C. and 74% relative humidity. In the malathion tests, the eggs were incubated for 20 days. In addition to the eggs that obviously had hatched, eggs on which the operculum was lifted and a nymph still within or partially emerged were considered to have hatched.

Discussion.—Chlordane is generally recognized as one of the most effective insecticides against cattle lice and it was used as the standard for comparison in these tests. Chlordane 0.5% normally provided 4 to 5 months of satisfactory control with a period of at least 2 months in which the herd was essentially lice-free. Chlordane at 0.25% was not quite as effective, and gave only 2 months' control in 2 of the 4 herds in which it was used. The treatments with chlordane and other insecticides seemed to give about the same period of control when applied in midwinter to heavily infested herds as when applied in early fall, while infestations were at a low level.

Heptachlor at 0.06% concentration or more, dieldrin at 0.03% or more, and malathion at 0.5% gave control

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Table 1.—Lice control on northern range cattle with residual sprays.

Insecticide	No. of Cattle Sprayed	Pretreatment Degree of Infestation[a]	Date Sprayed	No. of Animals Examined: Days After Spraying	Free of Lice	Lightly Infested[b]	Medium to Heavily Infested[b]	Days Satisfactory Control[c]
(1951–52)								
Chlordane 0.25%	34	Medium	1/11/52	8	6	—	—	
				53	2	3	1	53
0.5%	40	Medium	11/ 9/51	56	2	—	—	
				111	2	1	—	111+
0.5%	65	Medium	12/ 4/51	9	2	—	—	
				70	2	1	2	70
0.5%	63	Heavy	12/ 5/51	30	3	—	—	
				85	3	4	—	85+
Heptachlor 0.06%	83	Heavy	1/30/52	13	3	—	—	
				49	3	2	—	49+
0.06%	50	Light	12/28/51	7	3	—	—	
				82	3	1	—	82+
0.125%	55	Medium	12/13/51	22	2	—	—	
				49	4	1	—	
				96	1	4	—	96+
Dieldrin 0.125%	37	Heavy	12/27/51	35	5	—	—	
				82	3	1	—	82+
Lindane 0.046%	14	Medium	2/11/52	59	3	—	—	59+
Perthane 0.5%	75	Heavy	12/27/51	8	—	—	2	None
0.5%	31	Medium	1/29/52	5	1	—	3	None
(1952–53)								
Chlordane 0.25%	53	Medium	10/ 2/52	7	10	—	—	
				69	4	1	—	
				125	2	2	1	125
0.25%	168	Light	10/23/52	8	5	—	—	
				58	3	2	1	58
Heptachlor 0.06%	110	Light	10/ 2/52	6	10	—	—	
				63	6	1	—	
				156	2	1	2	156
0.06%	110	Medium	10/ 8/52	70	4	1	—	
				150	—	2	3	150
0.125%	101	Very Heavy	9/30/52	12	10	—	—	
				66	3	—	2	66
0.125%	102	Medium	10/22/52	7	5	—	—	
				56	4	—	1	
				135	1	2	1	135[d]
Dieldrin 0.06%	30	Light	10/ 7/52	64	5	—	—	
				119	3	2	—	119[d]
0.06%	230	Medium	10/17/52	55	5	—	—	
				110	—	5	—	110[d]
0.094%	90	Light	10/ 1/52	9	4	1	—	
				70	2	3	—	
				126	2	3	—	126+
Lindane 0.046%	76	Medium	10/22/52	15	5	—	—	
				56	2	—	3	56
(1953–54)								
Chlordane 0.25%	17	Light	11/13/53	187	3	—	—	187+
Chlordane 0.25%+	69	Medium	11/12/53	36	5	—	—	
Lindane 0.025%				152	—	1	4	152[d]
	80	Medium	11/13/53	47	4	1	—	
				112	2	1	1	112
Chlordane 0.25%+	87	Medium	12/ 2/53	28	2	2	—	
BHC 0.025% g.i.				100	4	1	—	
				161	4	1	—	161+
Dieldrin 0.03%	98	Light	11/11/53	37	5	—	—	
				114	3	1	1	
				192	2	2	1	192

[a] The following categories are used: light, no more than one animal out of 5 having lice at 5 or more/sq. in. in the most heavily infested area; medium, 2 or 3 animals out of 5 having 5 or more lice/sq. in. in the most heavily infested area; and heavy, at least 4 out of 5 with at least 5 lice/sq. in. in the most heavily infested area. Very heavy is used in three cases where all of the cattle examined had extensive areas solidly infested with lice. Some freedom was exercised in assigning these categories depending on extent of the heavily infested areas on the cattle.

[b] Lightly infested animals are those on which a total of fewer than 10 lice were found and less than 5/sq. in. in the most heavily infested spot. Those with more lice are rated medium to heavily infested.

[c] A + sign indicates that infested animals were so lightly infested, in some cases only 1 or 2 lice being found, that it was fairly safe to assume good control for at least 1 additional month.

[d] A few cattle, usually not more than 3 or 4, were missed in the roundup and not sprayed, or unsprayed cattle were introduced shortly after the herd was treated.

Table 1—(continued)

Insecticide	No. of Cattle Sprayed	Pretreatment Degree of Infestation[a]	Date Sprayed	No. of Animals Examined: Days After Spraying	Free of Lice	Lightly Infested[b]	Medium to Heavily Infested[b]	Days Satisfactory Control[c]
Lindane 0.03%	216	Heavy	12/ 1/53	22	3	1	1	
				101	—	—	5	−101
DDT 0.5%	31	Medium	11/11/53	37	4	1	—	
				121	2	2	1	121[d]
1954–55)								
Malathion 0.25%	100	Very Heavy	2/14/55	29	2	—	—	29+
0.5%	80	Very Heavy	11/24/54	6	5	—	—	
				126	—	5	—	126+
(1955–56)								
Chlordane 0.5%	190	Medium	10/19/45	64	5	—	—	
				140	—	5	—	140+
Malathion 0.5%	90	Medium	10/19/55	7	5	—	—	
				63	1	4	—	
				140	1	4	—	140+
Chlordane 0.25%+ malathion 0.5%	115	Light	10/18/55	4	5	—	—	
				142	4	1	—	142+[d]
Chlordane 0.5%+ malathion 0.25%	240	Medium	10/20/55	2	5	—	—	
				63	4	1	—	
				140	—	3	2	140
Chlordane 0.5%+ malathion 0.5%	250	Medium	12/20/55	78	4	1	—	78+[d]
Chlordane 0.5%+ malathion 0.5%	53	Light	10/18/55	66	5	—	—	66+[d]
Two Treatments (1952–53)								
Chlordane 0.25%	60	Light	10/ 1/52	99	5	—	—	
			10/21/52	158	1	2	1	158
Lindane 0.046%	46	Light	10/ 8/52	84	5	—	—	
			11/11/52	136	2	1	1	136
(1953–54)								
Dieldrin 0.03%	144	Heavy	11/12/53	79	4	—	1	
			12/16/53	154	2	2	1	154[d]

Table 2.—Ovicide tests with eggs of lice treated on cattle under field conditions.

Insecticide and Per Cent Active Ingredient	Egg Samples Taken	Per Cent Hatched	Per Cent of Nymphs Completely Extricated Before Death
Dieldrin, 0.03	Pre-spray	100	99
	1 hr. post-spray	62	45
	48 hr. post-spray	100	75
Chlordane, 0.25	Pre-spray	88	98
	1 hr. post-spray	87	64
	48 hr. post-spray	100	78
BHC, 0.025 g.i.	Pre-spray	100	99
	1 hr. post-spray	78	4
	48 hr. post-spray	68	7
Lindane, 0.025	Pre-spray	15	89
	1 hr. post-spray	38	0
	48 hr. post-spray	12	0
Malathion, 0.25	Pre-spray	64	—
	1 hr. post-spray	31	—

comparable to that with chlordane at 0.5%. Lindane and Perthane were inferior to the other insecticides used for lice control. BHC was not used in these tests, but it is used widely by ranchers because of its low cost per treatment. Since dieldrin probably has little possibility of use on cattle at any concentration level, heptachlor is the only one of the above insecticides that could compete with BHC on a cost basis. These two are considerably cheaper, cost-per-treatment, than other insecticides commonly used for lice control. For example, to treat 250 cattle with wettable-powder formulations at 2 gallons per head, it would cost $4.44 for BHC 0.03% gamma, $6.24 for heptachlor 0.06%, $15.20 for DDT 0.5%, $32.50 for chlordane 0.5%, and $68.00 for malathion 0.5% at current prices listed by a local formulator and distributor.

Laboratory tests with eggs of lice treated on cattle under field conditions with chlordane, dieldrin, BHC, and lindane showed that none of these insecticides killed many eggs although BHC and lindane at 0.025% killed more than 93 and 100%, respectively of the nymphs after the operculum was lifted and before they could extricate themselves from the egg shell. Wells & Barrett (1946) obtained 100% mortality of eggs by dipping them

for 1 minute in suspensions containing 1.0% and 0.1% gamma isomer of BHC. Furman (1947) found a high degree of ovicidal activity of BHC in laboratory-treated eggs, but found that many eggs hatched when they were clipped from BHC-treated animals several days after treatment. This, along with the data in table 2, indicates that BHC does not kill a high percentage of louse eggs under actual conditions of louse control although it is highly ovicidal at high concentrations in the laboratory.

A mortality of 52% resulted in eggs collected from a herd treated with malathion 0.25%. It was thought during the first 4 or 5 days of this test that the 0.25% malathion was going to give 100% mortality, as none of the treated eggs hatched. However, they began hatching by the end of a week, indicating a high mortality only in the older eggs. Cole & Burden (1956) have shown this to be the case with eggs of the body louse, *Pediculus humanus humanus* L. They found that with the phosphorus compounds the mortality became progressively higher as the age of the eggs increased, and at a concentration of 0.025% malathion caused mortalities ranging from 50.5% against 1-day-old eggs to 94.4% against 6-day-old eggs.

Malathion was not retested as an ovicide at a higher concentration, since field tests showed that when used alone at a concentration of 0.5% or when combined with chlordane, the control achieved was not longer than that obtained with chlordane alone at 0.5%. Better control may possibly have been achieved when the insecticides were used together, each at a concentration of 0.5%. This combination gave excellent control for the short observation period of 2 to 3 months, and might have exceeded the other sprays in effectiveness if observations could have been carried on over a longer period. The cost of such a combination spray would be prohibitive, however.

Two treatments with chlordane 0.25% and lindane 0.046% were better than one. Two treatments with dieldrin 0.03%, 35 days apart, apparently did not give control longer than one treatment, but the herd treated twice had the heavier infestation at time of treatment.

These results, together with results obtained by other workers, have led to the following recommended alternatives for cattle-lice control in Wyoming. In arriving at the recommendations, it was kept in mind that the average rancher does not do as thorough a job of application as was done in these tests.

a) Spray or dip, preferably twice, in October or November before onset of cold weather. It may be necessary to give a few animals individual treatment in late winter or spring.

b) Spray or dip once in October or November with a second treatment probably necessary about February. This has the advantage that the rancher may not need to make a second treatment.

c) If the herd is not available for treatment until December or January, treat once, and if thoroughly done, control should persist up to the time that lice populations normally decline in the spring.

Summary.—The period of residual effectiveness of chlordane, heptachlor, dieldrin, lindane, Perthane, DDT, and malathion for lice control on range beef cattle was determined by observing treated herds up to 6 months after single applications with wettable-powder suspensions. Chlordane 0.5%, heptachlor 0.06%, dieldrin 0.03%, and malathion 0.5% were about equal in effectiveness, in general providing 4 to 5 months of satisfactory control. This does not represent full seasonal control in Wyoming. The low cost per treatment of heptachlor, should it be approved for use on cattle, is an important point in its favor. Combinations of chlordane with BHC, lindane, and malathion did not prolong control. Lindane and Perthane gave control inferior to that obtained with other insecticides.

Chlordane, dieldrin, and lindane were not ovicidal under field conditions. Malathion 0.25% produced 52% mortality of eggs of lice and BHC 0.025% gamma isomer caused only a slight mortality under field conditions.

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Bioassay of Aldrin and Lindane in Soil[1]

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The rapid development and extensive use of organic insecticides has resulted in a need for suitable analytical techniques to determine insecticidal residues, particularly in foodstuffs. In many cases where chemical analytical techniques have not been adequate, relatively sensitive bioassay methods have been developed. Insecticidal residues have been determined using such insects as *Drosophila melanogaster* Meig. (Morrison 1945), *Musca domestica* L. (Laug 1946), *Tribolium confusum* Duv., *Tenebrio molitor* L. (Busvine & Barnes 1947), and *Aedes aegypti* L. (Hartzell & Storrs 1950).

Similar problems have resulted from the increased emphasis on soil application of insecticides. In addition to direct application soils may become heavily contaminated as a result of foliar applications. In many cases chemical analytical methods indicate the persistence of high concentrations of residues even after several years (Ginsburg & Reed 1954, Ginsburg 1955, Chisholm *et al.* 1950, Lichtenstein 1957). While this information is of considerable value, it is insufficient in itself, since it cannot be assumed that the material remaining in the soil is still insecticidally active. Chemical analytical methods may also be insufficiently specific (*e.g.* organic chloride), to detect chemical degradation that might alter or destroy insecticidal action. Furthermore the insecticide may become bound to some soil fraction in such a way as to become inactivated. Bioassay techniques on the other hand, give no information as to the nature of the insecticide present, but indicate the residual toxicity. In most past work the residue was assayed after it had been extracted from the soil with a suitable solvent (Fleming *et al.* 1951, 1953; Terriere & Ingalsbe 1953; Lauge & Carlson 1955). Very few results on direct soil bioassay have been reported (Fleming *et al.* 1951, Wylie 1956, Dewey, personal communication 1955). It would seem that to obtain maximum information concerning the persistence of an insecticide in a particular soil, chemical analysis should be used in conjunction with direct soil bioassay as well as bioassay of the extract.

Methods.—A test insect was required which would combine sensitivity to low concentrations of insecticide in soil or dry deposits with ease of rearing. Adult *Drosophila melanogaster* strain Cinnabar, fulfilled these conditions. Fruit flies were reared in half-gallon preserving jars on a pumpkin medium (Bartlett 1951). Two pounds of pumpkin puree plus 8 millilitres of propionic acid sufficed for three such cultures.

Tests were carried out in small jars, $2\frac{3}{4}$ inches in diameter and 3 inches deep, covered with fine screen inserted into the screw tops. When extracts were assayed, 1 milliliter of the extract and 0.5 milliliter of corn oil solution (1 milliliter corn oil in 200 milliliters hexane) were pipetted into each test jar. The jar was then slowly rotated until the solvent evaporated, so that the extract was deposited evenly over the interior of the jar. An extract of unknown concentration was tested against a range of standard concentrations. If preliminary tests gave too high a mortality, the unknown extract was suitably diluted with solvent. In cases where very exact estimates of the amount of insecticide present in an extract were required, several dilutions were used, and a curve plotted on log-probit paper parallel to the standard curve so that a relative potency determination could be made from LD_{50} values (Litchfield & Wilcoxon 1949).

In most of the work, a direct soil bioassay technique was used. Tests of *Drosophila* over an air dried soil containing insecticide gave erratic results, but the addition of an attractant into the soil counteracted this tendency. Of various fruit products tested as attractants, apple juice proved most satisfactory, giving a suitable smooth mixture with finely screened soil. The method was standardized as follows: Five grams of the soil under assay was weighed into each of two test jars. If preliminary tests indicated that this soil contained sufficient insecticide to bring it outside the range of the assay, less than 5 grams were weighed out, and untreated soil added to give a total of 5 grams. In each assay, at least four levels of standard were used, with two replicates per level. Ten milliliters of solvent were then added to all jars, and the appropriate amounts of insecticide in hexane solution were added to these standards. If the insecticide level in the test soil was expected to be low, then additional insecticide was added to the samples. The contents of each jar was well mixed, and the solvent was removed by standing the jars at the opening of a fume hood in a gentle air flow, care being taken to remove them from the flow as soon as all the solvent had disappeared. Three milliliters of apple juice were added to each jar, and thoroughly mixed into a slurry which evenly coated the bottom of the jar. Surplus moisture was evaporated off to give a firm surface. The high absorptive properties of some muck soils necessitated addition of 4 or even 5 milliliters of apple juice. To each test jar a small shell vial containing apple sauce was added to provide food.

Anaesthesis with either ether or carbon dioxide gave erratic results and a direct method of introducing the flies was employed (fig. 1). The stem of a small glass funnel was drawn out until the outlet was approximately 2 millimeters in diameter, such that the aperture was small enough to allow passage of only one fly at a time. Flies that had been isolated after emergence from pupae 3 days previous to the test were used. Up to one thousand such flies were collected into the reservoir jar and the funnel attached to the end of this by means of a screw cap. The funnel outlet was inserted into a small hole in the screen covering a test jar. With a bright light on the far side of the test jar, flies were counted as they passed down the stem of the funnel. When 50 flies had entered the jar, the funnel was withdrawn and a small cotton plug inserted into the hole. Pilot experiments showed 24 hours to be a suitable time to make mortality counts. By this time, the mortality/time curves had leveled off for both aldrin (12 hours) and lindane (22 hours) under the conditions of the experiments. Consequently, in all

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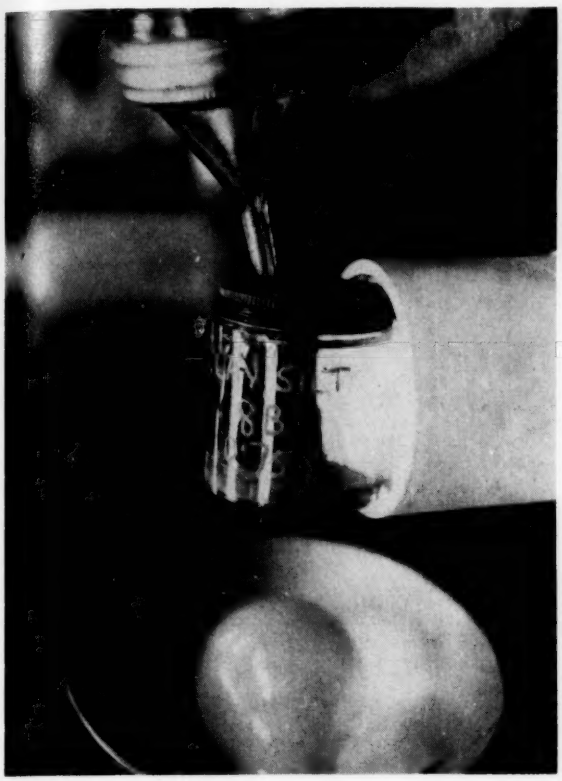

FIG. 1.—Introduction of flies into test jar.

experiments, mortality counts were made after a standardized period which was 24 hours of exposure to the insecticide. For each assay run, two controls were prepared and, if necessary, Abbott's correction for mortality in the controls (Healy 1952) was applied.

The effects of using flies of different age, sex, or strain were investigated in initial experiments. More consistent results were obtained with flies of a single sex, but the additional labor involved precluded this as a general practice. Significant differences in mortality between 1-day-old and 10-day-old flies were obtained, and also between flies of Wild and Cinnabar strains. In view of these results, all experiments were standardized on 3-day-old Cinnabar flies of mixed sex. During the course of the experiments it was noted that variations in such factors as light, temperature and humidity during the exposure period caused the results to vary appreciably. To eliminate this as far as possible, all experiments were performed under continuous light and under conditions of temperature and humidity which were nearly constant.

RESULTS AND DISCUSSION.—At an early stage in the work it became apparent that soils differed markedly in their properties as carriers of insecticide. Comparable mortalities of *Drosophila* were obtained in different soils only by the addition of large quantities of insecticide to some soil types. This appeared to be of considerable practical importance and was further investigated. From standard curves plotted for the three soil types; sand loam, loam, and muck, the LD_{50} values recorded in table 1 were obtained, and the ratios of these LD_{50} values to one another are shown in table 2.

Table 1.—Effect of soil type on the insecticidal action of aldrin and lindane.[a]

	ALDRIN (4 EXPERIMENTS)	
SOIL TYPE	Average LD_{50} (p.p.m.)	LD_{50} Range (p.p.m.)
Sandy loam	0.28	0.24–0.38
Loam	0.41	0.34–0.52
Muck	0.90	0.80–1.00
F value	103.70**	
L.S.D. @ 5% level	0.10	
@ 1% level	0.14	
	LINDANE (5 EXPERIMENTS)	
SOIL TYPE	Average LD_{50} (p.p.m.)	LD_{50} Range (p.p.m.)
Sandy loam	1.65	1.44–1.86
Loam	2.25	2.01–2.52
Muck	4.71	3.75–5.55
F value	29.95**	
L.S.D. @ 5% level	0.30	
@ 1% level	0.43	

[a] Test insect *Drosophila* with mortality counts after 24 hours.

A number of important observations can be made from these data. First, aldrin was more toxic to *Drosophila* than lindane. For each soil the LD_{50} for lindane was nearly five times as high as that for aldrin. This was not a soil factor, since a similar relationship was found when similar calculations were made from data obtained by the use of deposits of insecticide on the inner surface of test jars no soil being involved. The dosage/mortality curves (figures 2 & 3) had two interesting properties: curves drawn for aldrin had a greater slope than those obtained for lindane; and standard curves plotted for the same insecticide but in different soils were always parallel with the execption of those plotted for muck which had a lower slope with both aldrin and lindane. The ratios of LD_{50} values in different soils agreed closely for both insecticides (table 2) so that it seems probable that the adsorption of aldrin and lindane by a particular soil is due to the same factors.

Since these results indicated differential adsorption of insecticides by soils, the study was extended to include a wider range of soils, and an attempt was made to correlate the adsorption with physical properties of the soil. The most noticeable properties of soils showing the highest insecticide adsorption were their high organic matter content and high moisture holding capacity. To investigate the relative importance of these factors a range of 10 different soils was used. Estimations were made of the total organic matter content of each of these soils using the colorimetric method of Wilde & Petzer (1940). To obtain a relative value for the moisture holding capacity of each soil, samples were dried in a hot oven at 50° C. and a known volume of water was passed through a weighed amount of soil held in a filter funnel. The water which drained through was collected in a graduated cylinder for 1 hour. From the volume of water which drained through a particular soil, an estimate could be made of its relative

Table 2.—Ratio of LD_{50} values in different soils.

RATIO LD_{50} VALUES	ALDRIN	LINDANE
Sandy loam: muck	0.314	0.352
Loam: muck	0.456	0.478
Sandy loam: loam	0.683	0.734

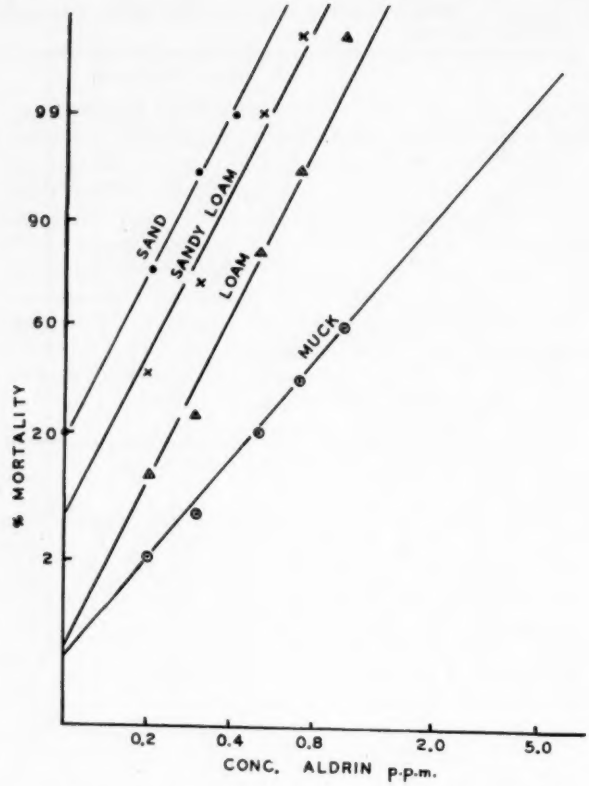

FIG. 2.—Dosage/mortality curves for aldrin in different soils.

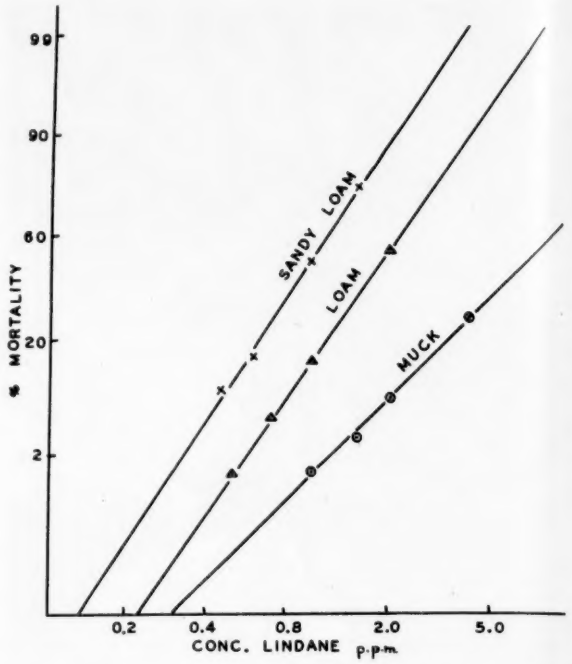

FIG. 3.—Dosage/mortality curves for lindane in different soils.

moisture holding capacity. Standard dosage/mortality curves were obtained for all these soils using both aldrin and lindane. With the exception of muck, the curves for all soils obtained with a particular insecticide were parallel. The LD_{50} for each insecticide in each soil was interpolated from the standard curve and tabulated with data on organic matter content and moisture holding capacity for that soil (table 3).

All these correlations were significant, but the correlation calculations were strongly weighted by the high correlation between organic matter content and moisture holding capacity, which existed in the clay loam and muck soils. When correlation coefficients were calculated omitting the data for these last three soils, significant correlation at 5% level was still obtained between the LD_{50} for both insecticides and the soil organic matter content. In contrast, correlation was no longer found between the LD_{50} values and moisture holding capacity of the soil. Thus it seemed probable that the important factor modifying toxicity of the insecticide in the soil was the organic matter present. Further support to this hypothesis was given by another experiment. Bentonite and Kaolinite are mineral soils containing negligible organic matter, but the former will absorb large quantities of water and the latter only a small amount. Standard dosage/mortality curves obtained in a similar manner for these soils showed no significant difference in LD_{50} value. This indicated that the moisture holding property was not the principal soil factor concerned with adsorption and retention of the insecticide in a non-toxic state.

The relationship between the LD_{50} values of lindane and aldrin and soil organic matter content is demonstrated in figures 4 & 5. It appears to be curvilinear, but could be considered as linear if muck is considered as exceptional. Before this can be finally decided, soils having organic matter contents intermediate between that present in the clay loam and the muck soils studied here, would have to be tested. Not many such soils are found naturally and none were available in the present work. There is some basis for considering muck as exceptional in its behavior to insecticides, in that all the standard curves obtained for muck differed in slope from those for other insecticides. It may well be that some additional factor present in muck accounts for the greatly increased adsorption. For practical purposes, *e.g.* the calculation of amounts of insecticide to be added to field soils, it seems probable that no great error would arise from considering the relationship between soil organic matter content

Table 3.—Relation between lindane and aldrin adsorption and soil factors.

Soil Type	Organic Matter (Per Cent)	Moisture-Holding Capacity (Per Cent)	LD_{50} of (24 Hours) *Lindane* (p.p.m.)	LD_{50} of (24 Hours) *Aldrin* (p.p.m.)
a. Springfield sand	0.5	51	0.25	0.055
b. Silty clay loam	1.0	71	0.38	0.175
c. Light sandy clay loam	1.2	52	0.51	0.065
d. Coarse silt	1.4	58	1.07	0.205
e. Silty clay	1.8	74	0.67	0.22
f. Sandy loam	2.6	49	1.25	0.22
g. Loam	3.8	83	2.65	0.34
h. Clay loam (lower level)	6.4	92	4.10	0.40
i. Clay loam (upper level)	10.0	—	5.9	0.54
j. Muck	40.0	127	8.6	0.85

Correlation coefficients
LD_{50} (aldrin) with organic matter content $r = 0.8935^{**}$
LD_{50} (lindane) with organic matter content $r = 0.9141^{**}$
LD_{50} (aldrin) with moisture holding capacity $r = 0.8468^{**}$
LD_{50} (lindane) with moisture holding capacity $r = 0.8204^{**}$

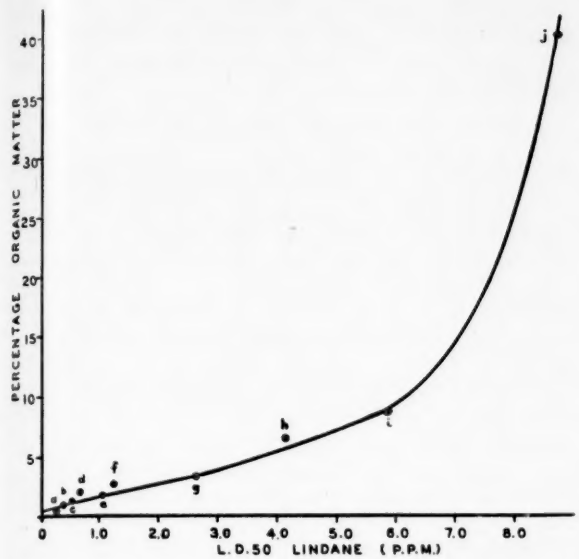

FIG. 4.—Relationship between soil organic matter and LD_{50} of lindane.

and adsorption of insecticide as linear, over the range of soils used in agriculture.

Plots of three typical, but very different, Wisconsin soils were treated with several dosages of aldrin and lindane. The amounts of insecticide applied and worked into these soils in the spring of 1954 were 20 and 200 pounds of aldrin per acre and 10 and 100 pounds of lindane per acre. These soils had been chemically analyzed at intervals to determine the dissipation of insecticide. With these data at hand, careful direct bioassays of the soils were carried out nearly 2 years after treatment. The assays were made on soil samples collected according to a random sampling procedure. Samples were diluted with untreated soil to five levels and measured against five

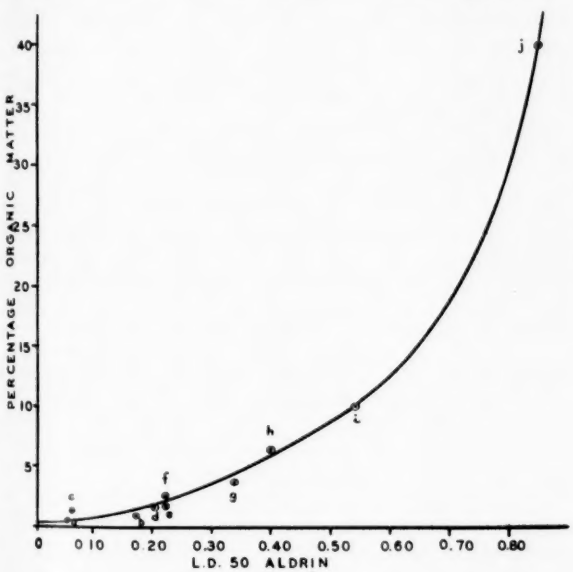

FIG. 5.—Relationship between soil organic matter and LD_{50} of aldrin.

Table 4.—Disappearance of lindane from soil 22 months after treatment.[a]

Soil Type	Treatment (Pounds/Acre)	Chemical Assay (p.p.m.)	Bioassay (p.p.m.)	Bioassay Confidence Limits (p.p.m.)	Bioassay / Chem. Assay ×100
Muck	10	11.6	2.21	1.87– 2.61	19
	100	118.6	28.4	24.1 –33.4	24
Loam	10	1.85	0.5	0.46– 0.56	27
	100	29.8	10.5	9.4 –11.7	35
Sandy loam	10	1.8	1.65	1.47– 1.85	90
	100	29.0	22.2	20.1 –24.0	76

[a] Treated May, 1954; bioassay, March, 1956.

levels of standard by the relative potency method (Litchfield & Wilcoxon). The results are shown in tables 4 and 5 together with data obtained by chemical assay of insecticide residues extracted from corresponding soil samples (Danish & Lidov, 1950) (Lichtenstein *et al.* 1956).

The considerable discrepancy between chemical estimates of residual lindane and the corresponding results obtained by bioassay is emphasized in the last column of table 4. It has been shown (table 1) that much more insecticide had to be added to a muck soil than to a sandy loam to obtain comparable toxicity to *Drosophila*. In each bioassay the insecticidal activity present in the assay soil was compared with that of standard concentrations prepared with soil of the same type. Hence, the estimate of the amount of insecticide present in a soil was independent of the immediate capacity of the soil to adsorb it and render it non-toxic. This was borne out by further experiments which traced the reduction in amount of insecticide present in a soil over a period of several weeks after application. Initially there was good agreement between the chemical and bioassay estimates of the amount of insecticide present, which indicated that the discrepancies observed in the later analyses were probably a feature of the time interval between application of the insecticide and the assay. If it is assumed that the extraction of the insecticide from the soil is efficient, the simplest explanation of the results is that degradation products of the gamma isomer lindane may have appeared. The bioassay recorded the actual residual toxic material while the chemical assay probably included degradation products of the insecticide which were no longer toxic (benzene ring structure). This would also account for the greater discrepancy between chemical and bioassay data seen with muck soil. The muck probably adsorbed the degradation materials of the insecticide just as efficiently as the insecticide itself.

Table 5.—Disappearance of aldrin from soil 25 months after treatment.[a]

Soil Type	Treatment (Pounds/Acre)	Chemical Assay (p.p.m.)	Bioassay (p.p.m.)	Bioassay Confidence Limits (p.p.m.)	Bioassay / Chem. Assay ×100
Muck	20	4.95	2.25	2.08– 2.43	45.5
	200	158.0	57.5	53.2 –62.0	36.2
Loam	20	0.69	1.89	1.75– 2.04	274.0
	200	16.5	28.0	26.0 –30.2	169.5
Sandy loam	20	0.24	1.25	1.15– 1.36	522.0
	200	22.5	66.0	60.5 –72.0	293.0

[a] Treated May, 1954; bioassay July, 1956.

Aldrin-treated soils were also assayed for residual insecticide 25 months after soil application. As in the case of lindane, there was considerable discrepancy between bioassay data and that obtained by chemical analysis. However, the estimates of toxic material present in loam and sandy soils made by bioassay were several times larger than the chemical results. This appeared to be the converse of the results with lindane (table 5).

The assay data indicated the possibility that aldrin was being degraded to a more toxic compound. In view of the report that aldrin may be readily oxidized to dieldrin (Bann 1956) samples were submitted to dieldrin analysis. These analyses showed that such a conversion had occurred in the treated soil. Furthermore the percentage conversion was greater for the soils having 20 pounds per acre applied to them than for those having 200 pounds. This showed close correspondence with the discrepancy between chemical assay data and bioassay results which were also greater in the soils having 20 pounds applied per acre. Additional confirmation came from later assays on the same soils. In these assays carried out 6 months after the first ones, there was greater divergence between bioassay and chemical data, which indicated that yet more aldrin had become converted to dieldrin.

CONCLUSIONS AND SUMMARY.—These preliminary studies indicate the value of bioassay as a tool in the study of soil insecticides. Chemical assays of soil insecticides cannot be complete in themselves, but require the additional information about residual toxicity which is provided by bioassay. Toxicity can be demonstrated by bioassay of the same extracts that are chemically analyzed. Direct bioassay of insecticide in soil can provide information on persistence and degradation of the insecticide. The degradation of the insecticide in the soil may result in a lower toxicity (*e.g.*, lindane) or increased toxicity (*e.g.*, aldrin).

Direct bioassay in soil also shows whether the insecticide becomes adsorbed in such a way as to reduce its toxicity. Such adsorption effects occurred with both aldrin and lindane and were correlated with the percentage of organic matter present in the soil. There was no indication of the exact nature of the interaction between the organic matter and the insecticide, but it appeared most likely to be an adsorption effect, since the insecticide could still be extracted in a toxic form. The reduction of toxicity with both aldrin and lindane was of the same order; it is probable that the adsorption is of a similar type for both insecticides. The direct soil method of bioassay provides quantitative data of the relative degree of insecticide adsorption by different soils. This may have extensive practical application since it is obvious that the soil type must be taken into account before the insecticide is applied in the field.

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Insectary Propagation of the Squash Bug and Its Parasite *Trichopoda pennipes* Fabr.[1]

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The larvaevorid fly *Trichopoda pennipes* Fabr., a parasite of hemipterous insects, apparently exists in the United States in at least three host-determined strains. One "strain" occurring in the eastern states attacks coreids, with the squash bug, *Anasa tristis* (DeG.), being its principal host. The second "strain," occurring in the south, attacks pentatomids, its major host being the southern green stink bug *Nezara viridula* (L.) (Beard 1940). The third apparent *T. pennipes* strain is found in California where it parasitizes the bordered plant bug, *Euryopthalmus cinctus californicus* Van Duzee, a pyrrhocorid (Gerhardt 1940). Thus, although *T. pennipes* has been known to occur in California for many years, it has never been recovered from *A. tristis* even though mixed populations of squash bug and parasitized bordered plant bug frequently occur in the field. This is contrary to the observations of O'Connor (1950) who found that a stock of *T. pennipes* obtained from Florida was indiscriminate in its attack upon mixed coreid, pentatomid, and pyrrhocorid species in Fiji. When caged with *A. tristis*, the California *T. pennipes* strain refuses to attack this host but when specimens of *E. cinctus californicus* are added to the cage the flies select out these individuals and oviposit on them exclusively. The reverse condition results when eastern (coreid attacking) *T. pennipes* are substituted for the western form.

Attempts were made to cross-mate the two strains of *Trichopoda* in the laboratory; however, no successful crosses were observed. In fact the flies showed little or no interest in the opposite sexes of the two strains.

Because of the absence of the coreid attacking *T. pennipes* from California, an attempt was made in 1953 to duplicate the earlier successful introduction of this strain into the Pacific Northwest (Clausen 1956). In this program it was hoped that a sufficient number of puparia could be collected in Connecticut to permit relatively large releases of the flies in California, thus eliminating the necessity of an insectary propagation program. Unfortunately, while in Connecticut the senior author did not encounter *Anasa* and *Trichopoda* in the numbers that had been anticipated, and plans for direct release of the flies had to be abandoned. Instead, the small stock of flies still living at the end of the 1953 season was utilized to oviposit on field-collected squash bugs, which were then held in a state of hibernation through the winter of 1953–54.

In the spring of 1954, it was planned to use the *Trichopoda* emerging from the overwintering stock to oviposit in field-collected squash bugs, in the hope of rearing parasites for release early in the summer. This plan also failed to materialize, however, for the bugs collected from the field in early spring were greatly weakened by their overwintering ordeal and succumbed quickly in the cages. Thus, instead of rearing large numbers of *Trichopoda* for release, the stock of parasites was barely maintained on the few hardy bugs that survived.

After this second failure to produce *Trichopoda* without resorting to a full-scale insectary rearing program, it was decided that the latter course would have to be adopted if substantial releases were to be made. The following discussion concerns the propagation program conducted in the summer of 1954.

METHOD OF REARING *Anasa tristis*—The main problem encountered in this phase of the program was the production of sufficient food for the squash bugs and making this food available to them in the insectary. The supply of food for this purpose was provided by a plot of pink banana squash, from which vine cuttings and fruits were obtained throughout the rearing program.

The basic rearing unit was a hot-wire tray developed by Flanders (1945) and later modified by him (unpublished data). Tray dimensions were 2′×4′×3″, and the hot resistance wire (45′ of 20L gauge) was coiled sufficiently to fit the inside of the top of the tray. This resistance wire produced enough heat to confine the nymphs without a cover and without burning the tray when connected to a 110-volt circuit. A cheesecloth cover 2′×4′ ×14″ was placed over the tray when the adults began to fly.

To initiate production on a rearing tray, *Anasa* eggs were obtained from a reproducing adult stock confined in a holding cage. This cage was simply a cheesecloth sack stretched over a wire frame, the open end of the sack being tied with twine. A single squash fruit was placed in each sack as food for the bugs, several hundred specimens being held in this manner. Bugs thus caged oviposited extensively on the cheesecloth, and eggs were readily obtained by cutting out patches of the cloth to which they adhered.

Vigorous squash vines, the cut ends of which were placed in water, were used to start the newly hatched nymphs, as they were unable to feed satisfactorily on fruits. Such vines remained in good condition on the trays for a week, if excessive numbers of bugs were not allowed to feed on them. When the vine began to collapse, either more vine or immature squash fruits were added to the trays. Eventually, the vines were eliminated from the rearing trays and the bugs were reared to maturity on the fruits. Single fruits supported large numbers of feeding bugs and suffered very little from molds or other factors causing rapid breakdown. The fruits were periodically replaced, however, as the feeding of the bugs finally caused them to shrivel and collapse.

Adult squash bugs appeared on the trays in from 25 to 30 days at the insectary temperature of approximately 78° F. From 800 to 1000 bugs were obtained per tray, and when fully hardened they were collected and placed in the cheesecloth sacks, where they were held until sexually mature.

Because of its reliance upon a field-grown source of food material, the rearing method described above was pos-

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sible only during the favorable squash-growing period. This, of course, was long enough to meet the needs of a propagation program of limited duration, but a method was needed which would permit year-round production of squash bugs. Such a method was developed by utilizing germinated squash seed instead of vines as a starter for the nymphs. Later, as the nymphs attained sufficient size, they were transferred to squash fruits and carried through to maturity exactly as described above. Since pink banana squash fruits can be held in storage for many months, a year-round food supply is available if enough fruits are stored.

In our program, seeds of banana or Hubbard squash were bedded in small hardware-cloth trays approximately $6'' \times 8'' \times 1''$ on a substrate of $\frac{1}{2}''$ moist sand over $\frac{1}{2}''$ of moist cotton. The cotton-sand substrate was kept moist by a cotton and cheesecloth wick from a $\frac{1}{2}$-pint glass fruit jar containing water. Seeds were germinated in a greenhouse at 80° F., the seedlings sustaining large numbers of feeding squash-bug nymphs for 10 to 14 days, sufficient time for them to reach the size at which they could be transferred to fruits.

METHOD OF REARING *Trichopoda*.—A successful method for rearing *Trichopoda* depended upon the development of (1) a sting or oviposition unit and (2) a holding unit where stung bugs could be held with minimum mortality, and where the parasite maggots could pupate successfully. In the development of the sting unit, two major problems had to be solved: (1) the minimizing of superparasitism (egg wastage) and (2) reduction of fly mortality resulting from the fluid ejected by the repugnatorial gland of the bugs. The major cause of superparasitism was found to lie in the tendency of a certain percentage of the bugs to congregate in the upper corners of the cage, where they were frequently stung excessively. Fly mortality, caused by the repugnatorial fluid of the bugs, was also related to the movement of bugs off the foodstuff onto the cheesecloth cover; for, as a rule, feeding bugs were not disturbed by the flies moving among them and did not eject their fluid to the same extent as those on the sides or top of the cage. The repugnatorial fluid acts much as a pyrethrum fly spray; *Trichopoda* contacted by the fluid immediately lose their sense of balance and move about erratically. Affected flies rapidly cease activity, the majority succumbing within 24 hours.

The sting unit giving the best results in eliminating bug wandering was the same hot-wire tray with cheesecloth covering frame as was used in rearing the bugs. This unit was provided with a squash-bug "food niche" composed of a single squash fruit and a section of fresh vine about 3 feet in length. The hot wire kept the bugs on the floor of the tray where the food attracted them. The flies settled on the cloth cover undisturbed by the bugs, fed through the cheesecloth cage top on sugar cubes and water-soaked cotton, and flew to the floor of the cage to oviposit.

Most efficient results were obtained from the sting unit when five female *Trichopoda* were placed with 200 to 300 squash bugs. At this parasite-host ratio, 30 to 50% sting occurred in 48 hours, with minimum superparasitism and generally low mortality to the flies.

In collecting stung bugs from the tray, the following procedure was adopted: (1) The flies were first collected from under the cheesecloth cover. (2) The fruits and vine with the bulk of the bugs feeding on them were then carefully lifted from the tray and placed in a 5-gallon lard bucket, and the bugs were anesthetized with CO_2. (3) Next, after the few specimens remaining on the tray were picked up and placed in the CO_2 bucket, the tray floor was washed and a new banana squash and section of vine were added. At the same time, paper towels were placed on the tray floor under the vine and fruit to absorb squash-bug fecal material and squash-fruit drippings. (4) Finally, the anesthetized bugs were quickly examined and the stung and unstung specimens segregated, the former being placed in the holding cage and the latter returned to the sting cage with a new stock of bugs. It was found that fewer *Trichopoda* were lost if the bugs were allowed to settle down before the flies were returned to the cage. By following the procedure described above, flies lived 3 to 4 days under optimum conditions and deposited an average of 20 eggs per female per 24-hour period.

The development of a holding unit for parasitized bugs posed additional problems which were only partially solved, as evidenced by the fact that 60% mortality of bugs occurred in the holding cages during the period. Inadequate nutrition appears to have been the primary mortality-producing factor, for it was found that bugs with developing maggots within them were weak and succumbed quickly when food was not kept fresh. To improve nutrition, many types of food were tried, including bouquets of seedling squash plants, rooted squash seedlings, and fruits of varying sizes and ripeness. It was eventually found that the best feeding media were large immature squash fruits or hardware-cloth trays of seedling squash.

The "stung-bug" holding unit was a cage $2' \times 1' \times 1'$ in dimensions, the bottom of which was $\frac{1}{4}''$ hardware cloth supported so as not to sag. This cage was placed over a metal tray containing a mixture of sand and peat moss about $\frac{1}{4}''$ thick over a $\frac{1}{2}''$ layer of moistened plaster of paris. The maggots, upon emerging from the dying bugs, dropped into the mixture of sand and peat moss, where they pupated. The puparia, collected at 2- or 3-day intervals, were kept for emergence on moist cotton in petri dishes in a cheesecloth-covered cage ($4' \times 2' \times 14''$). About 80% of the puparia in the emergence cage survived. A large percentage of the flies mated soon after emergence without manipulation, but to insure release of mated females, flies were paired in 4″ shell vials as suggested by O'Connor (1950). Generally, when flies were paired in this manner, mating occurred immediately, especially if older males were used.

RESULTS OF PROPAGATION EFFORTS.—Before discussing the quantitative results of this rearing program, it should be re-emphasized that the program was conducted during only one season and that a series of obstacles had to be overcome before satisfactory methods could be developed. Nevertheless, the writers are satisfied that maximum effectiveness in rearing squash bug and *Trichopoda* was approached by the end of the summer of 1954 and that if this program had been continued, vastly greater numbers of both host and parasite would have been produced.

Despite the difficulties encountered, approximately

10,000 *Anasa* and 2500 *Trichopoda* were reared during the summer. Since the sex ratio of *Trichopoda* was 1:1, some 1250 females were reared; of these, 551 mated individuals were released in three separate localities in California.

At all three release points the liberated females adapted themselves to field conditions immediately, and attacked their host with vigor. Stung squash-bug nymphs were observed 100 yards from a release point at Riverside, Calif., and in the main Riverside release area 25% of the squash bugs examined on October 10, 1954, bore *Trichopoda* eggs.

SUMMARY.—In the United States the larvaevorid fly *Trichopoda pennipes* Fabr. appears to occur as at least three host determined strains which are found in different geographical areas. In the summer of 1953, the coreid-attacking strain of the parasite was obtained from the squash bug, *Anasa tristis* (DeG.), in Connecticut, and shipped to California. Techniques were developed during the summer of 1954 for the insectary propagation of the parasite and its host.

In squash bug propagation, *Anasa* eggs were obtained from adult stocks held in cheesecloth sack cages with mature squash fruit as food. The newly hatched nymphs were fed cuttings of squash vine or rooted seedlings of squash grown on special trays. Squash fruits were later added to carry the nymphs through to maturity.

An oviposition unit was developed for production of *Trichopoda*, which minimized super-parasitism and reduced fly mortality caused by the repugnatorial fluid ejected by *Anasa*. Parasitized bugs were collected by anesthetization with CO_2 and placed in a holding cage. At this point, proper nutrition of the parasitized bug was found to be most critical. For this purpose, rooted squash seedlings or large immature squash fruit were found to be adequate. The puparia were collected from the moist sand under the holding cage and placed on dishes of moist cotton in a large cheesecloth emergence cage. A large percentage of the flies mated in the emergence cage, but to insure maximum mating flies were paired in 4″ shell vials.

During the summer of 1954, approximately 10,000 squash bugs and 2500 *Trichopoda* were produced, and of the latter, 551 mated females were released in three localities in California.

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The Effect of Prior Location on Alfalfa Foraging by Honey Bees[1]

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A successful method of increasing the collection of alfalfa nectar and pollen (especially the latter) by honey bees, *Apis mellifera* L., from colonies placed in or near alfalfa fields would be of great value in pollination. In some alfalfa-seed areas beekeepers shuttle colonies back and forth from one field to another. This practice is based on the belief that after disorientation bees will concentrate on the nearest available forage. To test the value of various types of colony movements in the management of honey bees for alfalfa pollination, we conducted a series of experiments in northern Utah from 1953 to 1955.

METHODS.—In each of three experiments three groups of colonies were used. One group had been placed in an alfalfa field near the beginning of bloom, the second was moved into the same field from an area where an abundance of bloom was available to the bees, and the third was moved into the field from a mountain location where alfalfa was not available and the bees presumably had no experience with alfalfa. Collection of data started the day after the second and third groups were moved into the field.

Two types of data were collected. (1) Pollen traps were placed under three to five hives in each group and samples of pollen were collected daily from each trap, and the amount of alfalfa pollen in the daily collections was determined. (2) Samples of 50 to 100 foraging bees were taken daily from the entrances of three to five other hives in each group, and examined to determine the presence of alfalfa pollen in the proboscis fossa, as described by Levin (1955) and Vansell (1955).

In each experiment an effort was made to select colonies of about equal strength. In 1955 the amounts of brood and pollen in each colony were measured to help explain differences in total amounts of pollen gathered.

LOCATIONS.—The experiments were conducted during July of 1953 and 1954 and July and August of 1955. In 1953 the alfalfa field to which the colonies were moved was on the Evans Farm, 3 miles south of Logan. About 10 acres of seed alfalfa were in bloom at the time, and varying amounts of hay alfalfa were blooming nearby. In 1954 and 1955 the colonies were moved to some seed acreage in Howell Valley, about 50 miles west of Logan.

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[3] The authors wish to express their appreciation to W. P. Nye for his help in carrying out some of the field work.

Table 1.—Total pollen and alfalfa pollen collected per colony by three groups of colonies in 14 days at the Evans Farm, Logan, Utah. 1953.

Group	Total Pollen (Grams)	Alfalfa Pollen	
		Grams	Per Cent of Total
Not moved	427	1.5	0.35
Moved from other alfalfa	782	4.3	0.55
Moved from mountain location	1076	13.6	1.26

Here at least 200 acres of alfalfa in bloom were available to the bees.

There was one striking difference between the two areas. The Evans Farm was located in an area of diversified farming. There were various attractive nectar and pollen sources, such as mustard, clover, and sweetclover, as well as Russian-thistle, corn, and unidentified plants. In Howell Valley the sources of pollen and nectar were much more limited and probably less attractive. Aside from alfalfa, the major sources were saltgrass, gumweed, Russian-thistle, and thistle. Sweetclover was present in small amounts.

At the Evans Farm alfalfa-pollen collection was practically nil, rarely by more than 1% of the bees seen in the field. In the alfalfa fields at Howell the proportion of bees observed collecting pollen sometimes exceeded 50%. Different sources of pollen vary in their attractiveness to bees, as do pure pollens when isolated from the plant (Levin & Bohart 1955). The greater proportion of pollen collectors at Howell can probably be ascribed to the less attractive complex of competing pollen sources. Apparently an equally important factor was the growing condition of the plants. Most of the alfalfa in seed fields at Howell appeared to be intrinsically attractive to pollen collectors.

POLLEN COLLECTION.—Data from the pollen-trap samples are presented in tables 1 and 2. The lower percentages of alfalfa-pollen collectors observed at the Evans Farm are reflected in the smaller percentages of alfalfa pollen in the traps.

The bees at Howell collected more pollen of all kinds in 1955 than in 1954. In both years both the total-pollen and the alfalfa-pollen collection tended to increase during the sampling period. The increase in amount of alfalfa pollen was maintained in spite of a decrease in the percentage. At the Evans Farm there were no consistent seasonal trends; alfalfa pollen was collected too sporadically and in too small quantities to warrant tabulation.

At both locations colonies moved from the mountains to the alfalfa fields collected about twice as high a percentage of alfalfa pollen (significant at the 1% level) as the other groups. At the Evans Farm, where these colonies collected more total pollen than either of the other groups, they also collected a significantly greater amount of alfalfa pollen. At Howell in 1954 the colonies

Table 2.—Total pollen and alfalfa pollen collected per colony by three groups of colonies in the Howell Valley, Utah. 1954 and 1955.

Date	Not Moved			Moved from Other Alfalfa			Moved from Mountain Location		
	Total Pollen (Grams)	Alfalfa Pollen (Grams)	Per Cent of Alfalfa Pollen	Total Pollen (Grams)	Alfalfa Pollen (Grams)	Per Cent of Alfalfa Pollen	Total Pollen (Grams)	Alfalfa Pollen (Grams)	Per Cent of Alfalfa Pollen
1954									
July 16	1.43	1.3	89	2.9	1.2	41	7.4	7.2	97
17	0.63	0.4	65	3.5	1.9	54	11.0	10.6	96
18	1.0	0.2	21	8.4	1.8	21	5.0	3.7	74
19	4.7	0.5	10	13.1	1.3	10	12.3	5.0	41
20	8.96	4.9	55	18.2	3.4	19	22.3	10.5	47
21	8.7	0.5	6	11.8	2.4	20	16.3	7.9	48
22	8.7	0.5	6	11.8	2.4	20	16.3	7.9	48
23	17.0	1.9	11	13.6	6.7	49	23.2	13.7	59
24	26.2	3.2	12	17.5	5.0	29	14.7	9.8	67
Total or Average	77.3	13.5	17.5	100.8	26.2	25.9	128.5	76.5	59.5
1955									
July 29	59	4.2	8	0	—	—	7	6.9	99
30	28	7.6	27	4	4.0	100	12	12.0	100
31	39	14.0	36	4	4.0	100	15	15.0	100
Aug. 1	64	26.9	42	3	2.9	97	28	25.5	91
2	64	26.9	42	3	2.9	97	28	25.5	91
3	78	50.8	65	5	5.0	100	38	36.8	97
4	93	65.2	70	7	4.1	59	40	34.2	86
5	89	42.5	48	15	5.5	37	54	41.9	78
6	89	42.5	48	15	5.5	37	54	41.9	78
7	116	48.7	42	23	7.5	33	84	65.9	78
8	116	55.3	48	22	8.6	39	74	58.8	79
9	102	48.0	47	22	7.6	34	71	57.6	81
10	171	75.7	44	42	12.3	29	107	76.1	71
11	73	43.0	59	18	7.2	40	42	33.9	81
12	137	84.5	62	29	9.8	34	82	60.6	74
Total or Average	1309	635.6	48.6	212	86.9	40.9	736	592.6	80.5

from the mountains also collected the most total pollen. Since the percentage of alfalfa pollen was greater for these colonies, the amount of alfalfa pollen was also significantly greater. In 1955 the colonies from the mountains collected more total pollen and far more alfalfa pollen than the ones from another alfalfa area (statistically significant at the 1% level), but only about half as much total pollen as those that had remained in the field. Consequently, they collected slightly less alfalfa pollen, even though the percentage was nearly twice as high.

ALFALFA VISITATION BY FIELD BEES.—The results obtained from examining the field bees caught at the hive entrances are shown in table 3. At the Evans Farm in 1953, where alfalfa was a minor source of nectar, the percentage of field bees visiting alfalfa for either nectar or pollen was much lower than at Howell, where alfalfa predominated.

At the Evans Farm the colonies moved from an area where alfalfa predominated visited alfalfa the most freely, and the group longest in the field visited alfalfa the least. At Howell the colonies longest in the field also tended to be lowest in alfalfa visitation. The colonies from the mountains showed the highest alfalfa visitation in 1954, but only about the same as the ones from another alfalfa area in 1955.

At the Evans Farm visitation to alfalfa by all groups of colonies tended to increase as the sampling period progressed. At Howell alfalfa visitation by the groups that had remained in the field stayed about the same, but for the other groups it showed a progressive decline in both years. By the end of the sampling periods the groups moved in were visiting alfalfa to a lesser degree than the ones that had remained there. However, none of these trends were statistically significant.

Table 3.—Per cent of field bees visiting alfalfa.

Date	Not Moved	Moved from: Other Alfalfa	Moved from: Mountain Location
		Evans Farm	
1953			
July 18	1.2	9.5	3.2
19	0.7	9.9	2.5
20	1.6	19.6	2.4
21	6.1	42.0	5.4
22	5.8	23.6	6.2
23	4.6	34.6	10.5
24	3.1	21.1	8.3
25	6.3	36.9	8.2
27	8.7	41.7	12.1
Average	4.5	27.7	7.0
		Howell Valley	
1954			
July 16	—	62.3	74.8
17	43.6	36.0	58.7
19	29.4	23.0	55.8
20	47.1	34.9	57.1
22	39.8	37.2	44.2
23	39.7	27.3	32.3
Average	39.9	36.8	53.8
1955			
July 30	39.8	67.2	68.9
31	39.0	55.1	62.4
Aug. 2	36.8	51.0	51.1
3	51.4	65.4	54.7
4	51.6	45.6	46.8
7	34.3	28.8	27.8
Average	42.2	52.2	51.9

BROOD AND POLLEN MEASUREMENTS IN 1955.—Four colonies from the stationary group averaged 239 square inches of pollen and 844 square inches of brood. Five colonies in the group from another alfalfa field had 395 square inches of pollen and 813 square inches of brood, while five colonies in the mountain group averaged 478 square inches of pollen and 1075 square inches of brood. The stationary group had 28% as much pollen as brood, while the other two groups averaged 48 and 44% as much pollen as brood, respectively.

DISCUSSION.—The data for the three seasons show that bees with no previous experience with alfalfa tend to collect a higher percentage of alfalfa pollen than colonies with such experience. This could be of considerable importance if the immigrant colonies always collected as much total pollen as the ones already accustomed to the area. Two seasons out of three this was true. However, in 1955 the stationary colonies, because of their high total-pollen intake, ended the sampling period with slightly more alfalfa pollen than the immigrants from the mountains.

The explanation for the differences between groups in total pollen collected is not known, but at Howell in 1955 there was evidence that colony condition, as indicated by amounts of brood and pollen in the combs, might have been partly responsible. While the amounts of pollen collected by the three groups were in accordance with their need, as expressed in the proportion of pollen to brood, the differences in pollen reserves hardly seem large enough to account for the great inequality in amount of pollen collected. Farrar (personal communication) reports that his observations do not show any correlation between pollen reserves and pollen collection. There is need for more investigations on factors within the hive that influence pollen collection.

As expected, the colonies moved from the mountains to the seed fields at Howell collected alfalfa pollen almost exclusively for a few days and then began to range more widely. Although the percentage of alfalfa pollen collected dropped off as a result, it didn't fall to the levels maintained by the other groups. Furthermore, because of a general rising trend in pollen collection, the total amount of alfalfa pollen collected actually increased during the sampling period.

The above trends reflect the generally increasing amount of bloom observed in the Howell Valley during late July and early August. This was even more noticeable for competing pollen sources than for alfalfa. The remarkable fact is that more bees began to collect alfalfa pollen in the face of increasing competition from other sources. The same phenomenon has been observed at Fielding and Newton, Utah. It appears that fluctuations in abundance of competing sources have little effect on alfalfa-pollen collection, except when the competing sources are extremely scarce.

The information obtained from field bees collected at the entrances indicates that the percentage of bees visiting a crop—at least for nectar—is roughly equivalent to the percentage that the crop comprises of the total

attractive bloom in the area. In Howell Valley, in both 1954 and 1955, about 45% of the field bees were working alfalfa, whereas on the Evans Farm in 1953 this figure was only about 13%. The proportion of alfalfa acreage in bloom in these two areas differed by about the same degree.

Under the conditions of more diversified flora at the Evans Farm, the stationary colonies were largely oriented to sources of nectar and pollen other than alfalfa. The bees moved into this diversified flora from an area where alfalfa was predominant carried with them their orientation to alfalfa for nectar. At Howell, where alfalfa predominated, all groups of colonies established and maintained a strong orientation to alfalfa. It appears, then, that moving colonies from one alfalfa field to another would be of special benefit to the second field only if alfalfa bloom was a major source in the neighborhood of the first field and a minor source near the second.

SUMMARY.—Experiments to determine the effect of prior experience with alfalfa on the activities of colonies of honey bees, *Apis mellifera* L., in an alfalfa field were conducted near Logan and Howell, Utah, in 1953, 1954, and 1955. Each year samples from pollen traps and from entrance-caught field bees were collected from three groups of colonies. One group had been in the alfalfa field from the beginning of alfalfa bloom in the area, the second had been moved in from another alfalfa field, and the third had been in an alfalfa-free mountain location for at least a month.

In all three years the colonies from the alfalfa-free mountain location collected about twice as high a percentage of alfalfa pollen as the other groups. In 2 years they also collected greater quantities of all pollen and alfalfa pollen. In the third year they collected less than half as much total pollen and a slightly smaller amount of alfalfa pollen than the group that had remained in the field.

The percentage of entrance-caught field bees working alfalfa was roughly equivalent to the percentage of alfalfa bloom in the area.

Results of these experiments indicate that moving colonies from one alfalfa field to another will be of special benefit only when the move is made from an area where alfalfa is predominant to an area where alfalfa comprises only a small part of the bee pasturage.

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Tests with Insecticides for Control of Cotton Insects in the Lower Rio Grande Valley[1]

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For several years field-plot tests have been conducted near San Benito, Tex., in the Lower Rio Grande Valley with different insecticides, applied alone and in combinations, to determine the best control measures to use for the boll weevil (*Anthonomus grandis* Boh.) and other cotton insects that frequently occur at the same time, such as the pink bollworm (*Pectinophora gossypiella* (Saund.)), the bollworm (*Heliothis zea* (Boddie)), the cotton aphid (*Aphis gossypii* Glov.), the cotton fleahopper (*Psallus seriatus* (Reut.)), and different species of spider mites (*Tetranychidae*). The tests discussed herein were conducted in 1948, 1950, and 1952–54.

METHODS.—Two series of tests were conducted in 1948 and 1950 and one in each of the other years. Most of the work was with dusts, but sprays were also used. The plots were arranged in randomized blocks and ranged in size from $\frac{1}{4}$ to $\frac{1}{2}$ acre for the dust experiments and from approximately $\frac{2}{5}$ to 1 acre for the sprays. Each series consisted of three to eight replicates. The applications were made at 4- or 5-day intervals insofar as possible, the dusts being applied with rotary hand guns and the sprays with knapsack sprayers. Those washed off by rain within 24 hours were repeated as soon as time would permit. The average number of applications made in the different series ranged from five to nine.

The infestation records were made at about weekly intervals. Yield records were obtained for some of the tests. The insecticides used, infestation counts, and yields are summarized in table 1.

TESTS IN 1948.—In series 1 all the treatments gave satisfactory reductions of the boll weevil populations, the greatest being from the chlordane plus sulfur. The best increase in yield was obtained from the calcium arsenate plus sulfur, this being closely followed by all the other treatments except the chlordane plus sulfur. Although the chlordane-sulfur treatment gave the best control of the weevils, it seemed to have had an adverse effect on the plants, and consequently a lower yield was obtained than for the other treatments.

In series 2 all the treatments with calcium arsenate, alone and in combination with sulfur and parathion, gave about equally effective control of weevils and practically the same substantial increases in yield. BHC plus DDT and sulfur and BHC plus methoxychlor and sulfur gave only fair reductions. A substantial gain in yield was obtained from the BHC-DDT sulfur treatment, whereas the increase was noticeably lower for the BHC-

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Table 1.—Seasonal infestation averages for the boll weevil and bollworm, and yields following treatments with various dusts and sprays at San Benito, Tex., 1948, 1950, and 1952–54.

Treatment	Pounds of Toxicant per Acre	Boll Weevil Infestation (Per Cent)	Per Cent of Bollworm-Injured Squares	Pounds of Seed Cotton per Acre	
				Total	Gain
Tests in 1948					
Series 1, dusts (5 plots, 6 applications):					
Check	—	56.8	—	2151	
Toxaphene 20%+sulfur	3.2	26.0	—	2412	261
BHC, gamma 3%+DDT 5%+sulfur	0.50+0.84	32.6	—	2435	284
Chlordane 10%+sulfur	1.6	17.4	—	2329	178
Calcium arsenate+sulfur 1:1	8.8	23.0	—	2444	293
Calcium arsenate	10.6	25.2	—	2405	254
Series 2, dusts (5 plots, 6 applications):					
Check	—	62.2	4.1 (4.4)[a]	1518	
BHC, gamma 2%+DDT 10%+sulfur	0.30+1.5	39.7	0.7 (0.3)	2060	542
BHC, gamma 2%+methoxychlor 10%+sulfur	0.33+1.7	40.2	8.2 (11.4)	1760	242
Calcium arsenate+sulfur 1:1+parathion 0.25%	7.96+0.04	27.8	3.4 (3.3)	2045	527
Calcium arsenate+parathion 0.5%	8.46+0.04	26.4	3.6 (2.9)	2054	536
Calcium arsenate	9.4	25.7	1.3 (1.8)	2046	528
Tests in 1950					
Series 1, dusts and sprays (8 plots, 9 applications):					
Check	—	68.2	—	—	
Toxaphene 20%+sulfur		33.1	—	—	
Dieldrin 2.5%+sulfur		19.1	—	—	
Dieldrin spray 0.375 to 0.50		21.5	—	—	
Calcium arsenate+sulfur 1:1		37.6	—	—	
Series 2, dusts (4 plots, 8 applications):					
Check	—	83.2	—	—	
Low-lime calcium arsenate+parathion 1%	10.1+0.10	33.3	—	—	
Low-lime calcium arsenate+BHC, gamma 1%	12.18+0.12	32.3	—	—	
Heptachlor 5%+sulfur	0.99	16.2	—	—	
Aldrin 5%+sulfur	1.0	25.1	—	—	
Calcium arsenate+sulfur 1:1	9.9	39.6	—	—	
Tests in 1952					
Sprays (3 plots, 5 applications):					
Check	—	65.4	54.2	—	
Metacide 0.375–0.75	0.53	40.1	39.0	—	
Methyl parathion 0.375–0.75	0.53	35.3	31.2	—	
Heptachlor 0.375–0.75	0.53	52.1	32.2	—	
Endrin 0.375	0.375	11.3	0.5	—	
Dieldrin 0.375	0.375	13.2	2.7	—	
Tests in 1953					
Dusts (7 plots, 7 applications):					
Check	—	62.3	10.9	443	
Heptachlor 2.5%+sulfur	0.37	37.5	10.0	686	243
Heptachlor 5%+sulfur	0.87	30.0	11.2	597	154
Methyl parathion 2.5%+DDT 5%	0.44+0.87	32.5	5.2	834	391
Low-lime calcium arsenate+methyl parathion 1%	10.49+0.11	18.7	6.8	830	387
Dieldrin 2.5%+sulfur	0.43	28.8	7.1	743	300
Tests in 1954					
Dusts (7 plots, 5 applications):					
Check	—	46.1	—	2366	
Low-lime calcium arsenate+methyl parathion 1%	12.08+0.12	26.5	—	2484	118
Endrin 1.5%	0.28	23.5	—	2594	228
Dieldrin 2%+methyl parathion 1%+DDT 5%	0.33+0.17+0.84	28.1	—	2631	265
Dieldrin 2.5%+sulfur	0.41	24.1	—	2537	171

[a] Figures in parentheses indicate per cent of injured bolls.

methoxychlor-sulfur mixture because of the damage from bollworms.

TESTS IN 1950.—The yields in 1950 may not be strictly comparable; therefore they have been omitted and the results will be discussed from the standpoint of control or reduction in the boll weevil populations.

In the first series dieldrin dust and spray gave good control of the boll weevil infestations, whereas the reductions for toxaphene plus sulfur and calcium arsenate plus sulfur were noticeably lower. Toxaphene was included in a spray at 3 pounds per acre, but it failed to give satisfactory results after three applications and its use was discontinued. The dieldrin spray was the only spray used throughout the season. It gave good control of the

boll weevil, but the plants did not seem to fruit as well as where the dieldrin-sulfur dust was used.

In the second series all the treatments gave substantial reductions of boll weevils, the heptachlor-sulfur giving the greatest reduction and calcium arsenate-sulfur the least.

TESTS IN 1952.—In one spray series endrin gave very good control of both the boll weevil and the bollworm, and this was followed closely by dieldrin. Methyl parathion gave only fair control of the boll weevil under heavy infestations late in the season. Metacide did not give quite as good control as methyl parathion, and heptachlor was the poorest of all the materials. Satisfactory control of the bollworm was not obtained with methyl parathion, Metacide, or heptachlor. Aphids were held in check by the methyl parathion, Metacide, and endrin. None of the treatments was effective against the pink bollworm. No yield records were obtained.

TESTS IN 1953.—In one dust series the best reduction of boll weevils was obtained with low-lime calcium arsenate containing 1% methyl parathion. All the other treatments gave satisfactory control, except the 2.5% heptachlor and sulfur, where the control was only fair.

Bollworms caused some injury in this series. The damage was the greatest in the heptachlor plots, and the least where DDT was used with methyl parathion.

No appreciable control of the pink bollworm was obtained from any of the treatments except where the DDT was included with the methyl parathion.

Cotton fleahoppers were causing considerable damage at the time some of the tests were started, but the first poison application practically eliminated the infestations in both the treated and untreated plots.

The greatest increase in yield was from the methyl parathion plus DDT. This was followed closely by the low-lime calcium arsenate plus methyl parathion, the dieldrin-sulfur being the third best. Damage from bollworms was responsible in part for the yields not being higher for the two heptachlor treatments.

TESTS IN 1954.—In one dust series all treatments gave satisfactory control of the boll weevil under moderate infestations. However, none of the treatments showed much promise against a light infestation of pink bollworms.

The best increase in yield was from the dieldrin-methyl parathion-DDT mixture, and the next from endrin. It is doubtful, though, if the 1.5% endrin would be satisfactory for heavy infestations of the boll weevil. Spider mites may have been responsible for the yields not being a little higher for the endrin treatment.

The Reproductive Capacity of Three Strains of the Two-Spotted Spider Mite Complex[1]

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Although great differences in susceptibility to acaricides have been reported among strains of the spider mites *Tetranychus telarius* (L.) and *cinnabarinus* (Boisd.), no morphological differences have been found for distinguishing resistant and nonresistant mites of the same species (Smith & Fulton 1949, 1951, Taylor & Smith 1956). However, general observations have indicated that mites in colonies of the different strains vary in the rate of increase in population under greenhouse laboratory conditions. Between December 1955 and May 1956 experiments were conducted at Beltsville, Maryland, to compare the oviposition rate and development of mites from three strains of this spider mite complex and the changes in the populations following the mixing of strains. These strains, designated as the nonresistant Massachusetts and resistant Cranbury-1 colonies of *telarius* and the carmine nonresistant Beltsville colony of *cinnabarinus*, were the same as used in earlier experiments on the transmission of resistance (Taylor & Smith 1956).

In these studies the mean greenhouse temperature was 75° F., the daily averages ranging from 69° to 83°, and the mean relative humidity was 40% with a daily range of 28 to 68%. The mites were reared on the primary leaves of lima bean plants, which were grown in pots until ready for use and then cut at the ground level and placed in bottles containing a balanced nutrient solution, where they formed roots. To prevent the mites from wandering off the plant, a barrier of lanolin, which is nontoxic to plant tissue, was wiped around each petiole. Another barrier of Deadline, a long-lasting but phytotoxic sticky preparation, was also applied around the neck of each bottle. These barriers proved completely effective in retaining the females. Only a few males were lost, though they had a strong tendency to wander off soon after reaching adulthood, especially when no females were present. The barrier method of confining the mites to uncaged leaves, similar to that used by Boudreaux (personal communication, 1956), was preferred to the celluloid strips and cells used by Cagle (1949) or jars used by Iglinsky & Rainwater (1954), because it did not alter the temperature and humidity.

In order to insure observation from the beginning of the egg-laying period, female mites were transferred to the prepared leaf by means of a fine sable-hair brush while in the deutonymph stage. An adult male transferred to the leaf at the same time would usually "stand guard" beside the quiescent stage, into which the deutonymph transforms by the next day, and assist her in casting her skin when she emerges a day later. Mating takes place immediately after emergence of the adult female. However, as previously reported (Perkins 1897, Cagle 1949), mating was observed among older mites. One virgin Massachusetts female, which had laid only unfertilized eggs from which only males developed, produced female

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offspring after a male was placed on the leaf with her on the 16th day. The male was usually left with each female for 1 day. All females fertilized during the first day as adults laid both fertilized and unfertilized eggs throughout their egg-laying period. Furthermore, fertilization had apparently no effect upon the rate or number of eggs produced, for three unfertilized mites, one Cranbury and two Massachusetts, averaged the same number of eggs as those females that were fertilized.

In all three colonies egg laying did not start until about 2 days after the adult female emerged. The eggs were usually laid within depressions in the under surface of the leaf, often along the veins. Females of the Cranbury and Massachusetts colonies of *telarius* tended to lay their eggs in a smaller area than the *cinnabarinus* mites.

Boudreaux (1956) uses the color of the egg as one of the features separating *telarius* from *cinnabarinus*. However, when first laid, eggs of all three strains in our experiments were observed to be colorless and translucent, those in both *telarius* colonies turning to opaque and whitish after a few hours. On the third day they became a greenish-buff or straw-colored with a gradual shading to a light rust area on one side, whereas most of the *cinnabarinus* eggs became wine-tinged with clearly defined wine spots. However, a number of the *cinnabarinus* eggs turned a cloudy greenish-straw color with wine or rust spots, thus making them indistinguishable from *telarius* eggs represented by the Cranbury and Massachusetts mites.

Under our greenhouse conditions the eggs of all three colonies hatched either late on the fourth or early on the fifth day after being laid. The newly hatched larvae were straw-colored for several hours and then changed to green. The protonymphs and deutonymphs were also green. Females of *cinnabarinus* in the Beltsville colony did not assume the carmine color typical of this species until the second day after becoming adults.

Reports in literature indicate a lack of agreement as to the number of instars in the male. McGregor & McDonough (1917) and McGregor (1950) reported only one nymphal stage in the male and two in the female, while Cagle (1949) and Iglinsky & Rainwater (1954) reported two nymphal stages in both exes. In the present study a single six-legged larval and two eight-legged nymphal stages were observed in both males and females of all strains. The three molts took place at 2-day intervals, approximately 1 day being required for each active and 1 for each quiescent stage. Adult females emerged on the eleventh day after the egg had been laid, males one-half to a full day earlier.

In one series of tests single females of each colony were isolated on separate leaves and moved to new leaves every 1 to 3 days. In a second series five females per leaf were used as a unit and similarly moved. In both sets of observations there were outstanding differences in egg production between the three colonies of mites. In the group of isolated females the average number of eggs produced per day was 5.0 for Beltsville mites, 7.3 for Cranbury mites, and 9.4 for the Massachusetts mites (table 1). The respective maximum numbers of eggs laid during a life span were 122, 153, and 212.

Table 2 summarizes the results in the second group containing 5 mites per unit. Here likewise the differences are highly significant. These differences are further accentuated by the fact that a peak in production is reached in each case between the sixth and ninth days of the oviposition period, the difference being highly significant in Cranbury and Massachusetts but not in the Beltsville strain.

More females than males were produced by fertilized females in each strain (table 3). The deviation from an expected normal 1:1 ratio was highly significant, as determined by chi-square analysis.

No great variation was observed in the life span, which averaged between 17.6 and 20.5 days, or the length of the oviposition period, which averaged slightly more than 2 weeks in all strains tested. The *cinnabarinus* colony showed a greater tendency to produce nonviable eggs, but no definite conclusion could be reached from this limited study.

In connection with these life-history studies, experiments were conducted to determine the changes occurring in a mite population where two strains were placed to-

Table 1.—Reproduction by isolated females of three strains of spider mites.

Strain	Parent Life (Days)	Oviposition Period (Days)	Number of Eggs			Number of Adults Emerged	
			Total	Nonviable	Per Day	Females	Males
Beltsville	20.5	15.2	76.5	19.8	5.0	43.8	11.5
Cranbury	17.6	14.8	106.8	4.6	7.3	68.7	31.7
Massachusetts	20.4	14.7	134.1	4.0	9.4	64.4	27.8

Table 2.—Reproduction by groups of five females in each of three strains of spider mites.

Age of Parent (Days)	Average Number of Eggs per Female per Day		
	Beltsville	Cranbury	Massachusetts
0–2	3.0	4.7	12.7
2–4	3.7	6.0	10.0
4–6	2.7	5.3	7.8
6–9	5.3	10.3	15.7
9–13	3.7	7.3	9.3
13–18	2.7	8.3	4.7
Mean	3.5	7.0	10.0
L.S.D. For strains (5%)	1.1	(1%)	1.5
For daily egg production per female (5%)	1.5	(1%)	2.1
Total number of eggs per female	54.3	101.0	143.8

Table 3.—Proportion of sexes in progeny of fertilized female spider mites.

Strain	Progeny		Chi-Square (1:1 Ratio)	
	Females	Males	Computed	Required for 0.01% level
Beltsville	261	54	27.2	13.3
Cranbury	275	127	13.6	11.3
Massachusetts	290	124	22.2	9.2

Table 4.—Changes in proportion of resistant to nonresistant female mites in experimentally mixed populations.

Days After Mixing	Resistant and Nonresistant *telarius*		Resistant *telarius* and Nonresistant *cinnabarinus*			
	Mites Examined	Per Cent Resistant	Mites Examined	Per Cent Resistant *telarius*	Per Cent Resistant Hybrid	Per Cent Nonresistant *cinnabarinus*
0	20	50	20	50	0	50
41	408	46	327	47	24	29
49	—	—	317	32	51	17
60	100	24	121	20	73	7
84	162	24	319	38	51	11
91	911	49	1788	60	35	5

gether on the same plants. On December 9, 1955, a group of bean plants were infested with 10 females each from the nonresistant Massachusetts and the resistant Cranbury colonies. Likewise 10 females each of *cinnabarinus* (Beltsville) and resistant *telarius* (Cranbury) were placed on another group of plants. Fresh plants were provided during the following 3 months as the mites required food. At varying intervals during this period samples of mites from each population were tested for resistance by dipping in a 1–5000 malathion emulsion (Taylor & Smith 1956). Although the Massachusetts mites had a higher egg-laying rate than the Cranbury mites (tables 1 and 2), the results in table 4 show that resistant and nonresistant *telarius* mites were approximately equal in numbers at the end of the experiment. In the mixed population of resistant *telarius* and *cinnabarinus* hybridization increased during the first 60 days, but as the *cinnabarinus* mites declined the *telarius* mites became more abundant.

This preliminary experiment indicates that resistance in a mite population would not be lowered by adding an equal number of a more prolific strain of the same species. Also, negative results are indicated in the tests to produce sterile hybrids through mixing with a closely related species.

The revision of the *telarius* complex by Boudreaux (1956) is amply supported by his described differences in morphologic characters. That *telarius* and *cinnabarinus* are distinct species is further confirmed by the fact that, although interbreeding of these two species does produce hybrid females, they are mostly sterile. In Boudreaux's experiments (1956) 164 hybrids were closely watched, and only four of them laid eggs, nine of which hatched and developed into males although the four females had mated. Taylor & Smith (1956) failed to get any viable eggs from carefully isolated hybrid females. The highly significant differences in the reproductive capacities of the two strains of *telarius* and *cinnabarinus* mites observed in this study support the recent reclassification of this mite complex.

With reference to our two strains of *telarius*, A. E. Pritchard, E. W. Baker, and H. B. Boudreaux failed to disclose any morphologic differences between the resistant Cranbury and the nonresistant Massachusetts mites. Their close relationship was further confirmed by Taylor & Smith (1956), who obtained fertile females from crosses between these two strains. In the present studies no differences were found between these strains in the incubation periods of the eggs or the rate of development of the immature mites. The significantly higher oviposition rate of nonresistant Massachusetts over that of resistant Cranbury mites is a biological difference between two strains of the same species, but may not necessarily apply as a generalization for other strains, whether resistant or nonresistant.

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The Effect of the Protozoan Parasite *Perezia pyraustae* Paillot on the European Corn Borer[1]

HAROLD L. ZIMMACK[2] and TOM A. BRINDLEY[3]

In a report by Zimmack *et al.* (1954), the distribution of *Perezia pyraustae* Paillot, a protozoan parasite of the European corn borer (*Pyrausta nubilalis* (Hbn.)) and its effect upon the host were discussed. Additional information on the effect of the parasite on the corn borer is presented herein.

The study was divided into three parts: (1) laboratory experiments with stock reared on a synthetic food medium through one complete generation, (2) longevity and egg production of female moths, and (3) histological evaluation of infection in corn borers in various stages of development.

LABORATORY TESTS WITH REARED STOCK.—*Methods.*—A synthetic food medium developed by Beck (1950) was used in the laboratory rearing experiments. This medium was composed of a bacto-agar carrier to which adequate nutritive constitutents had been added. The medium was stable and did not require renewal during larval development. An aseptic technique developed by Beck & Stauffer (1950) was employed to minimize contamination.

During larval development, the corn borers usually fed on the surface of the medium and were in contact with the wall or bottom of the rearing vials.

In July and August 1955 breeding stock was obtained by collecting summer-brood larvae and pupae in Polk County, Iowa. Seven egg-laying females were reared from this material. All of these were found through examination of eggs deposited and post mortem examination to be infected with *P. pyraustae*. Approximately 30% of the eggs in an egg mass laid by an infected female were infected. Thus, all diseased corn borers used in this study were naturally infected transovarially and could be compared with uninfected larvae that had hatched from the same egg mass.

All rearings were done in constant temperature cabinets at 30±2° C., and a relative humidity of 70±3%.

Only egg masses in the blackhead stage were used. The masses were placed in a 2% solution of a commercial 1-to-300 trypsin preparation and held at 35° C. with agitation every 10 minutes. In approximately 45 minutes the eggs became separated. The eggs were then taken up in a medicine dropper and transferred through six changes of distilled water to remove the trypsin. Eggs settled at the tip of the vertically-held medicine dropper, and a number of them could be transferred in a single drop. The eggs were transferred from the last water rinse into 40% ethyl alcohol for 1 minute, then into distilled water again. They were surface-sterilized by transferring them to a solution containing 2% sodium hydroxide and a 2% formaldehyde. After the eggs had been in the sterilizing solution for 10 minutes, they were transferred to 70% ethyl alcohol in an aseptic transfer chamber, which contained ½-by 2-inch cotton-stoppered vials with synthetic food medium. Each egg in a drop of the alcohol was then dropped into one of the vials. The drop of 70% alcohol did not appear to have an adverse effect upon hatching. The mouth of the vial was flamed over an alcohol lamp, and the cotton plug was then replaced.

RESULTS.—A total of 360 hatched first-instar larvae were reared on an uncontaminated synthetic food medium. Larval survivals are summarized in table 1. Nineteen days after hatching all of the infected group and all of the uninfected group except three larvae had reached the fifth instar or pupal stage. Survival was 12% higher in the uninfected group than in the infected group at this time. During the course of the rearing experiments, every dead borer was dissected and examined microscopically to determine if *P. pyraustae* was present or absent.

Subsequent study of the corn borers reared on a synthetic medium was carried on. A final comparison of the two groups shows that of the original 305 uninfected larvae, 234 or 77% developed into adults whereas of the original 55 infected larvae, 32 or 56% developed into adults. Thus, survival was 21% higher in the uninfected group.

Data relevant to longevity and egg production were recorded for 70 pairs of moths obtained from rearings on a synthetic medium. Infected females averaged 7 days of life and produced an average of 83 eggs. Uninfected females averaged 10 days of life and produced an average of 122 eggs. An average of 18 eggs out of 83 (22%) were infertile in the infected moths, whereas an average of 33 out of 122 (27%) were infertile in the uninfected moths. These data are presented in table 2. Evidently the synthetic medium and/or the artificial procedure of rearing and pairing the moths reduced the egg production and caused a high rate of infertility. These rearing experiments showed that, as in field conditions, first-instar larvae infected with *P. pyraustae* can develop into moths that can mate and produce fertile eggs.

LONGEVITY AND EGG PRODUCTION.—*Methods.*—Each captured pair was caged in individual oviposition chambers in the laboratory for egg laying. The egg masses and the moths, after their death, were examined for *Perezia*. Rearings were conducted at 30±2° C. and a relative humidity of 70±3%. A total of 336 moths (172 females and 164 males) and 2,774 egg masses were examined. Data on longevity and egg production of 172 paired female moths collected while mating in large emergence cages are also summarized in table 2. *Perezia*-infected moths compared with uninfected moths averaged 3 days shorter life, 10 fewer egg masses, and 47% fewer eggs. More than half of the egg masses from infected moths showed infection. An average of four eggs in 160 were in-

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Table 1.—Cumulative data for nine experiments on the development and survival of European corn borer larvae reared on synthetic medium.

	Uninfected		Infected	
	Number	Per Cent Survival[a]	Number	Per Cent Survival[a]
Eggs:				
Laid	470		73	
Hatched	369		62	
—Contaminated vials	−64		−7	
Hatched larvae in uncontaminated vials	305		55	
Surviving larvae:				
3 days after hatching				
1st instar	82		3	
2nd instar	222	99.7	49	94.5
5 days after hatching				
1st instar	12		1	
2nd instar	56		6	
3rd instar	232	98.4	45	94.5
7 days after hatching				
1st instar	6		0	
2nd instar	6		1	
3rd instar	64		12	
4th instar	216	95.7	39	94.5
9 days after hatching				
1st instar	2		0	
2nd instar	5		0	
3rd instar	11		1	
4th instar	163		26	
5th instar	111	95.7	24	92.7
11 days after hatching				
2nd instar	6		0	
3rd instar	6		0	
4th instar	29		5	
5th instar	248	94.8	45	90.9
13 days after hatching				
2nd instar	4		0	
3rd instar	3		0	
4th instar	11		2	
5th instar	265	92.8	46	87.3
15 days after hatching				
2nd instar	1		0	
3rd instar	3		0	
4th instar	5		1	
5th instar	218		31	
Pupae	52	91.5	15	85.5
17 days after hatching				
2nd instar	1		0	
4th instar	4		1	
5th instar	132		27	
Pupae	139	90.5	18	83.6
19 days after hatching				
2nd instar	1		0	
4th instar	2		0	
5th instar	94		18	
Pupae	173	88.5	24	76.4

[a] Survival is based on larvae which hatched and were reared on uncontaminated synthetic food medium.

fertile in eggs laid by infected females whereas only an average of one egg in 301 was infertile in eggs laid by uninfected females. An egg was determined to be infertile if no trace of embryological development could be observed. It was not determined whether the infertility was due to *Perezia*, lack of fertilization, or other causes.

The factors responsible for the low production of uninfected eggs from infected females was not determined. It is interesting to note that these data compare almost identically with the earlier study made on longevity and fecundity by Zimmack *et al.* (1954) in which infected females averaged 48% fewer eggs than uninfected females.

A total of 23 diseased male moths copulated with disease-free females. *P. pyraustae* was absent in all egg masses laid by the females, indicating that the diseased male does not play a part in the transmission of the disease through copulation.

HISTOLOGICAL EVALUATION OF HOST SYSTEMS ATTACKED.—*Methods.*—The purpose of the histological work in this study was to determine the systems of the corn borer attacked by *P. pyraustae*, the manner in which transovarial transmission of the parasite is accomplished, and how the infection spreads during the development of corn borers. Healthy and diseased corn borers prepared for histological evaluation were obtained from egg masses laid by infected female moths. As in previous rearing experiments, all stages of corn borers were developed on a synthetic food medium.

European corn borers in all stages of development, except oviposited eggs, were sectioned. Sections of oocytes were obtained from the reproductive organs of female moths. All material was fixed in Gilson's fluid for approximately 2 to 3 hours. The extreme anterior and posterior ends of the body were severed or pinholes were made in the body wall of all larvae after fixation. Paraffin infiltration was slightly better in the specimens in which the ends of the body had been severed. The *n*-butyl alcohol method of Becker & Roudabush (1939) was employed. Parlax with a melting range of 54° to 56° C. was used for imbedding. The specimens remained in Parlax for approximately 20 days. Stiles (1934) emphasized that long periods of infiltration (minimum of 2 weeks) are absolutely necessary. Embedded blocks of tissues were trimmed and placed in water containing a slight amount of detergent. After being in the water for a minimum of 3 days, the tissue was sectioned.

Heidenhain's iron haematoxylin staining technique, as described by Becker & Roudabush (1939), showed nuclear details of the host tissue and *P. pyraustae* spores. The Ziehl-Neelsen and methylene blue staining technique, described by Lillie (1948), was effective for the determination of infected organs. Masses of *P. pyraustae* spores were stained red by the carbol fuchsin and stood out prominently from the uninfected host tissue which was stained blue by the methylene blue A clear contrast between infected and non-infected areas was shown.

More than 600 slides of histological sections were made of 92 European corn borers in different stages of development. Fifty-two of the specimens were infected and 40 were uninfected. The uninfected specimens were useful in studying internal anatomy. Whenever possible, five slides were made from each specimen. In each series of slides, for any given specimen, one or two were stained with Ziehl-Neelsen, and the remaining ones were stained with iron haematoxylin. Methylene blue was used as a counterstain with Ziehl-Neelsen, and eosin was used with iron haematoxylin. Sections cut at 4 or 5 microns usually gave the best staining results with either of the two stains. Sections at 3 microns or below were usually too thin to show cellular details.

RESULTS.—Histological observation of infected ovaries revealed that nurse cells were most often the chief site of infection, figure 1. The germarium and follicular epithelium were also infected. *Perezia* spores were present in the material that passes from the region of the infected nurse cells into the developing oocyte. Masses of the spores occurred in the yolk of many oocytes.

In a study of four diseased first-instar larvae, three important facts were noted. First, *Perezia* spores were present in the nervous system of two of the larvae. Second, the parasites occurred in the digestive epithelium and

Table 2.—Effect of *Perezia pyraustae* on longevity and egg production of European corn borer moths.

Group of Moths	Number of Moths	Days from Mating to Death		Number of Egg Masses Laid				Number of Eggs Laid		Number of Infertile Eggs	
				Total		Total Infected					
		Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
70 paired female moths reared on synthetic food media, 1955											
Infected moths that laid—											
Any eggs	5	3–11	6.8	3–17	7.2	0–3	0.8	13–244	82.8	3–46	18.4
Infected eggs	2	3–4	3.5	3–4	3.5	1–3	2	13–66	39.5	9–16	12.5
Uninfected eggs	3	8–11	9	5–17	9.7	0	0	37–244	111.7	3–67	22.3
Uninfected moths that laid eggs all uninfected	65	4–21	9.9	1–57	10	0	0	3–839	121.5	0–273	32.8
172 paired female moths collected in Polk County, Iowa, 1955											
Infected moths that laid—											
Any eggs	149	2–21	9.6	1–56	14.9	0–48	9.7	1–617	160.2	0–65	4.2
Infected eggs	134	2–21	9.5	1–56	16.3	1–48	10.9	4–617	176.1	0–65	4.5
Uninfected eggs	15	3–15	10.5	1–7	2.5	0	0	1–75	18.9	0–19	1.5
Uninfected moths that laid eggs all uninfected	23	2–22	12.9	2–60	24.0	0	0	7–789	301.2	0–6	1

lumen of the digestive tract in three of the larvae. Third, only one of the larvae had infected Malpighian tubules. Since the Malpighian tubules are usually the chief site of infection in late instar larvae, it is probable that infection spreads to these organs from other diseased parts of the corn borer. Evidently, the cytoplasm was replaced by the *Perezia* spores. Only in the case of the heavily infected Malpighian tubules were the cell membrans damaged. The pressure of the protozoan in the host cytoplasm caused lesions and allowed masses of spores to pass into the lumen of the Malpighian tubules.

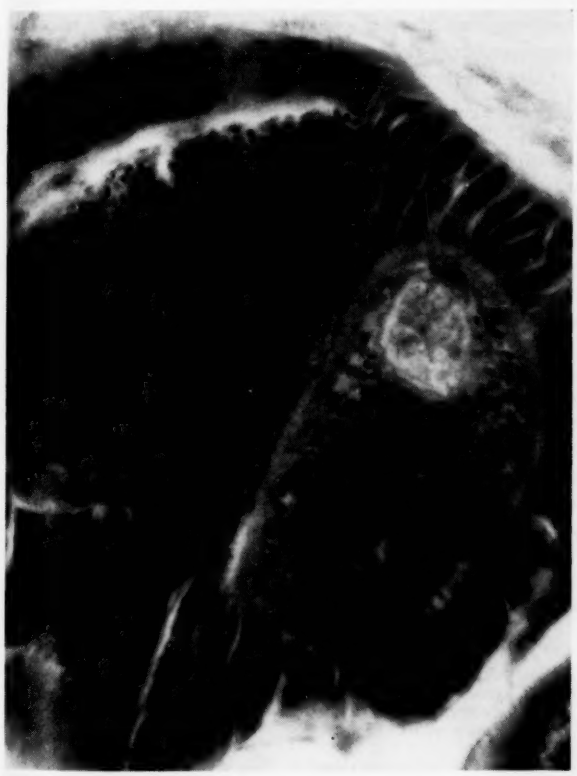

FIG. 1.—Cross section of ovary through region of nurse cells. *Perezia* spores are heavily concentrated in the cytoplasm of the three nurse cells.

In study of 48 infected specimens (first through fifth instars) the Malpighian tubules were infected in 38. The nervous system, mainly the cellular area (cytons), was infected in 31 of the specimens. The digestive tract was infected in 26 and the silk glands in 14 specimens. Other tissues were infected less frequently. The integument was infected in 8, the muscles in the fat body in 4 and the salivary glands in 3 of the larvae. Spores were also observed in the haemocoel. In 10 of 28 moths, the reproductive structures were infected.

SUMMARY AND CONCLUSIONS.—The protozoan parasite *Perezia pyraustae* Paillot is an intracellular parasite that infects all stages of the European corn borer (*Pyrausta nubilalis* (Hbn.)).

A total of 360 hatched first-instar larvae were reared on uncontaminated synthetic food medium. In the diseased group 56% developed into adults as compared with 77% of the uninfected group. Dead corn borers were dissected and examined microscopically to determine whether or not *P. pyraustae* was present. Emerged male and female moths were paired and egg production and longevity data were recorded. Infected moths averaged 3 days shorter life and produced an average of 39 less eggs than uninfected moths. Although mortality was 21% higher in the diseased group, the rearing experiments showed that as in field conditions, larvae infected with *P. pyraustae* can develop into moths that can mate and produce fertile eggs.

Egg production and longevity data on copulating moths showed that the *Perezia*-infected moths compared with the uninfected moths averaged 3 days shorter life, nine fewer egg masses and laid 47% fewer eggs.

Histological evidence of transovarial transmission of the parasite was demonstrated in the ovary of the corn borer. *P. pyraustae* spores were shown passing from the region of infected nurse cells into the developing oocyte. Diseased males did not play any part in the transmission of the disease through copulation with uninfected females.

The Malpighian tubules were the chief site of infection in the host. Usually infection in these organs was absent or light during the first and second-instar stages but increased rapidly in subsequent development. The digestive tract was infected in more than half of the infected specimens.

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Toxicity of Certain Insecticides for Control of Pale Western Cutworm Attacking Wheat in Kansas[1]

L. J. DEPEW[2] and T. L. HARVEY[3]

The pale western cutworm, *Agrotis orthogonia* Morr., is a serious pest of wheat and other small grains in the Great Plains region. This insect caused severe damage to dryland winter wheat in northwestern Kansas during the spring of 1956.

The cutworm is a subterranean insect that burrows through the soil. According to Sorenson *et al.* (1941), the cutworm feeds on the wheat plants from about $\frac{1}{4}$ to 1 inch below the soil surface. Usually a short section of the underground portion of the stem is either partly or wholly eaten through. Damage is soon apparent at the surface by wilting and eventual death of the plants. Because of the insect's subterranean nature, it is not so readily controlled by poisoned baits and surface sprays as are most other cutworms. The present study was conducted to compare the effectiveness of certain insecticides in controlling the pale western cutworm.

Chemical control investigations have been conducted recently by several workers. Jacobson *et al.* (1952), found that dieldrin (8 oz.) and chlordane (32 oz.) were the most effective materials tested. Aldrin, BHC, toxaphene, and heptachlor proved unsatisfactory. Hoerner (1953) found that 1.5 lbs. TDE, 8 oz. dieldrin, and 8 oz. aldrin gave sufficiently high mortality to be considered practical controls, while toxaphene, DDT, and chlordane appeared to be less effective. Pfadt (1956) obtained complete control with endrin at 0.2, 0.3, and 0.4 lb. per acre, good control with endrin (0.1 lb.), TDE (1.0 and 2.0 lbs.), or dieldrin (0.4 and 0.5 lb.), but poor control with heptachlor (0.5 lb.) or toxaphene (2.0 lbs.) at the end of 16 days.

MATERIALS AND METHODS.—Portions of two infested wheat fields located in northwestern Kansas were divided into four blocks of seven plots each (test 1) and eight plots each (test 2). Each plot was 60×60 feet. Six treatments and one check (test 1) and seven treatments and one check (test 2) were assigned at random to each block. The insecticides tested and their rates of application included aldrin and heptachlor at 1 lb., endrin at 0.25 lb., dieldrin at 0.5 lb., and TDE, DDT, and toxaphene each at 2 lbs. per acre. All sprays were made from emulsifiable concentrates. Application was made with a gear-pump, tractor-mounted sprayer equipped with an 18½-foot boom having hollow cone-type nozzles spaced 20 inches apart. All treatments were applied at a pressure of 40 p.s.i. and at the rate of approximately 6 gallons per acre. Boom height of 24 inches gave complete coverage of the ground. Treatments were applied April 19 and 25, 1956, for test 1 and 2, respectively. The wheat was approximately 6 inches tall at the time of application. After spraying, one third of each plot was rotary-hoed and the remaining portion left undisturbed.

Cutworm populations before and after treatment were determined by sifting 10 randomly selected soil samples in each plot. Each sample measured 1-square-foot and was approximately 6 inches deep. Sifting was by means of a portable dry soil sifter which sorts by shaking the soil through two trays: a top tray with a 2-mesh screen and a bottom tray with an 8-mesh screen.

Although cutworm populations varied within the fields, pre-treatment sampling revealed no significant variation in infestation throughout the test area. Therefore, percentage control was based on differences in live cutworm counts between treated and untreated plots 7 and 14 days after application. Cutworms were mainly fourth and fifth instar larvae at the time of treatment.

Climatological data recorded between treatment and final sampling 7 days later in test 1 showed that the maximum temperature ranged from 48° to 87° F. with an average of 66° F. The minimum temperature range was 30° to 42° F. with an average of 35° F. Precipitation measured 0.02 inch. In test 2, the climatological data between treatment and final sampling 14 days later indi-

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Table 1.—Effectiveness of six insecticides 7 days after application by ground sprayer for control of the pale western cutworm in winter wheat. Edson, Kansas, April, 1956.

Treatment	Pounds of Toxicant per Acre	Rotary-Hoed		Uncultivated	
		Mean No. Cutworms[a]	Per Cent Control	Mean No. Cutworms[a]	Per Cent Control
DDT	2.0	2.0	81	2.5	77
Endrin[b]	0.25	2.5	76	3.0	73
Dieldrin[b]	0.50	4.5	57	4.5	59
Heptachlor[c]	1.0	5.0	52	5.5	50
Toxaphene	2.0	5.7	46	8.2	26
Aldrin[b]	1.0	6.0	43	9.2	16
Check	—	10.5	—	11.0	—

[a] Average number per four replications.
[b] Supplied by Shell Chemical Company.
[c] Supplied by Velsicol Chemical Corporation.

cated that the maximum temperature ranged from 38° to 92° F. with an average of 67° F. The minimum temperature range was 25° to 48° F. with an average of 37° F. Precipitation measured 0.05 inch.

Results.—*Test 1.*—The relative effectiveness of the insecticides tested is summarized in table 1. In this test, DDT was the most toxic material used, giving 81% kill in the rotary-hoed portion and 77% kill in the uncultivated portion. Endrin was next in order of effectiveness, giving 76% kill in the cultivated portion and 73% kill in the uncultivated area. Toxaphene and aldrin gave the lowest percentage control, with 46 and 43%, respectively, in the rotary-hoed portion and 26 and 16%, respectively, in the uncultivated portion. Control in the remaining treatments ranged from 52 to 57% in the cultivated area and 50 to 59% in the uncultivated areas. However, dieldrin, aldrin, heptachlor, and toxaphene did not give satisfactory control of the cutworms.

Test 2.—Results of test 2 are summarized in table 2. Of the materials tested only TDE, DDT, and endrin were effecting a high degree of mortality 7 days after treatment. TDE was the most toxic material, giving 94% kill in the rotary-hoed portion and 90% kill in the uncultivated portion. However, DDT and endrin were not greatly inferior to TDE in their initial toxicity to cutworms. Heptachlor, aldrin, dieldrin, and toxaphene were relatively ineffective at the dosages used. All materials, with the exception of toxaphene, gave increased mortality 14 days after application.

Discussion.—It appeared evident from this investigation that TDE, DDT, and endrin gave excellent control. Since the data exhibited many zero counts, it was generally not suitable to analysis of variance. However, chi-square tests showed that these treatments were significantly better than the other treatments tested. Heptachlor, aldrin, and dieldrin failed to give practical control in test 1 and did not give satisfactory control until 14 days after application in test 2. Toxaphene gave poor results in both tests. Although heptachlor, aldrin, dieldrin, and toxaphene were significantly inferior to the three best materials, chi-square tests showed that they were significantly better than the checks.

This study confirms the results obtained in Wyoming (Pfadt 1956) which showed that endrin and TDE were superior to heptachlor and toxaphene. However, the good control obtained with DDT in these tests did not agree with the results obtained in Colorado by Hoerner (1953) in which he found DDT inferior to aldrin, dieldrin, and TDE.

Working the soil after spraying appeared to cause slight increases in the effectiveness of the insecticides. However, control obtained with TDE, endrin, and DDT were satisfactory without working the soil.

Dieldrin, aldrin, and heptachlor gave a substantial increase in mortality at 14 days compared with 7 days in test 2. This would seem to indicate a slower lethal action in some cases. Just how long these materials will keep the cutworm too sick to feed is unknown. This is an important factor because 7 additional days of larval feeding may cause severe crop losses to the wheat farmer.

Summary.—Experiments were conducted in northwestern Kansas during 1956 to evaluate the effectiveness of TDE, DDT, endrin, dieldrin, aldrin, heptachlor, and toxaphene against the pale western cutworm, *Agrotis orthogonia* Morr., infesting winter wheat. The materials were applied as emulsion sprays by means of a gear-pump, tractor-mounted sprayer. Cutworm populations before and after treatment were determined by using a portable dry soil sifter.

Table 2.—Effectiveness of seven insecticides applied by ground sprayer for control of the pale western cutworm attacking winter wheat. Goodland, Kansas, April, 1956.

Treatment	Pounds of Toxicant per Acre	Control in Rotary-Hoed Area After Days Indicated				Control in Uncultivated Area After Days Indicated			
		7		14		7		14	
		Mean No. Cutworms[a]	Per Cent Control	Mean No. Cutworms[a]	Per Cent Control	Mean No. Cutworms[a]	Per Cent Control	Mean No. Cutworms[a]	Per Cent Control
TDE[b]	2.0	2.2	94	0.0	100	2.5	90	0.0	100
DDT	2.0	2.5	93	0.5	98	3.0	88	0.2	99
Endrin	0.25	2.7	93	0.2	99	2.5	90	0.0	100
Dieldrin	0.50	10.7	71	5.5	76	9.2	68	1.5	93
Aldrin	1.0	12.2	67	1.0	96	7.5	70	3.7	82
Heptachlor	1.0	13.7	63	1.0	96	10.7	57	2.5	88
Toxaphene	2.0	20.7	44	13.0	42	14.0	43	11.5	43
Check	—	36.7	—	22.5	—	24.7	—	20.2	—

[a] Average number per four replications.
[b] Supplied by Rohm & Haas Company.

TDE and DDT at 2 lbs. and endrin at 0.25 lb. actual toxicant per acre gave excellent reductions in cutworm populations. Aldrin at 1 lb., dieldrin at 0.5 lb., and heptachlor and toxaphene at 1.0 lb. actual toxicant per acre decreased populations significantly when compared with the untreated check but were inferior to the other three treatments and failed to give practical control within 7 days after application.

Working the soil after spraying slightly increased the effectiveness of the insecticides in most cases, but seemed unnecessary since some insecticides effected good control in undisturbed soil.

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Factors Influencing Pink Bollworm Winter Carryover in Central Texas[1]

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Heavy infestations of the pink bollworm (*Pectinophora gossypiella* (Saund.)) in central Texas in recent years have shown the need for more information on the biology and habits of the pest, especially the major factors influencing its carryover from one cotton crop to the next.

When they have completed feeding, many pink bollworms become inactive for a long period. These inactive larvae have been called resting, overwintering, long-cycle, or diapause larvae to distinguish them from the short-cycle type, which pupate and emerge soon after they complete feeding.

Investigations were started at Waco, Tex., in November 1952 to determine the winter carryover in several environments, seasonal occurrence of the resting stage, and time and rate of emergence. The more important findings up to October 1956 are briefly discussed herein.

METHOD.—To determine winter survival and the time and rate of emergence of the pink bollworm from hibernation, a screened-wire pyramid-type cage, with a removable cover, as described by Shiller (1946), was used. Infested open cotton bolls or the fruiting forms of various wild or cultivated plants were placed inside the cages in the late summer or fall.

Moth emergence from open cotton bolls was observed in four environments—namely, (1) above ground to simulate standing plants and buried 2 inches on February 15, (2) on the soil surface and buried 2 inches on the same date, (3) buried 2 inches on November 15, and (4) on the soil surface throughout the experiment.

Three and one-half pounds of infested open bolls were placed in each cage on November 15 of each year. There were 20 cages per treatment in 1952, 1953, and 1954, and 15 cages in 1955. After the bolls were thoroughly mixed, 10 to 20 samples of 1 pound each were taken at random from each collection and examined to determine the number of larvae installed per cage. The number of larvae in each environment ranged from 5,676 to 12,513 during the various years. The removable covers, equipped with glass-jar moth traps, were placed on the cages on March 31 of each year, and moth emergence was recorded almost daily until completed.

To determine the seasonal occurrence of the resting stage during the growing season, mature larvae were removed from hard green bolls at semimonthly periods and placed in petri dishes, where they were permitted to spin cocoons under thin disks of absorbent cotton, according to a method described by Fife (1949). This method permitted observations without disturbing them. The dishes were kept in an insectary under approximately outside temperature and humidity conditions. Larvae were considered in the resting stage if they did not pupate within 30 days.

SEASONAL OCCURRENCE OF THE OVERWINTERING STAGE.—Part of each brood that developed in green bolls entered the overwintering stage. Overwintering larvae were present in all samples (table 1). In green bolls 11% or less were present during July and August of both years, whereas a rapid increase occurred in September. In October and November 93% or more entered the overwintering stage. In 1955 resting larvae per pound of open bolls showed essentially the same trend.

These data suggest that the date of stalk destruction is an important factor in limiting the number of overwintering larvae that could survive to infest the next crop. To be materially effective it must be done before September 15, although this is too early in the season to be of practical use. However, stalk destruction as soon as possible after harvest would be of value, since it would reduce the late-season buildup during a period when most of the larvae enter the over-wintering stage.

Fife *et al.* (1947) showed that, in the Lower Rio Grande Valley of Texas survival to infest cultivated cotton in open bolls installed in cages on August 8 to 16, September 11 to 18, and October 12 to 19 averaged approximately 0.4, 5, and 25%, respectively. Based on these studies a mandatory stalk-destruction date of August 31 for that area has given effective control of both the pink bollworm and the boll weevil.

RELATION OF ENVIRONMENT TO SURVIVAL AND EMER-

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Table 1.—Seasonal occurrence of resting larvae of the pink bollworm in central Texas.

Period	Per Cent from Green Bolls		Number of Larvae per Pound of Open Bolls (1955)
	1955	1956	
July 16–31	—	6	7
August 1–15	3	10	—
16–31	5	11	—
September 1–15	25	33	18
16–30	64	75	—
October 1–15	93	96	198
16–31	94	98	—
November 1–15	100	—	238

GENCE.—The time and rate of moth emergence from hibernation in four environments, based on a 4-year average, are shown in figure 1.

There were two major peaks of emergence, and they occurred at about the same time in each environment. The first peak occurred between approximately April 22 and May 10 and the second peak from about May 22 to June 10 (fig. 1, B). A greater percentage of the moths emerged during the second peak from bolls installed on the soil surface (not buried), but the opposite was true with respect to the other environments. Also, smaller peaks occurred in all environments within 2 to 3 weeks after 0.5 inch or more of rainfall.

Fruiting forms began to develop on the earliest planted cotton in this area about May 15. Therefore, only moths that emerged after this date were able to infest cultivated cotton. As shown by the vertical lines in figure 1, *A*, approximately 25, 54, 62, and 67% of the total emergence had occurred by this date in the various environments. These data show that burial of infested bolls increased the rate of emergence and consequently a larger percentage emerged before cotton was available for propagation.

In a separate series of cages, the first emergence occurred on March 17 in 1955 and the last on August 24, or a maximum emergence period from overwintering larvae of about 5 months.

Table 2 shows the winter survival of the pink bollworm in various environments for each of the 4 years. Winter survival was higher in bolls on the soil surface and lower in bolls buried in November than in any of the other environments. There was no significant difference between the two February-burial treatments.

A winter survival of 6.2% occurred in the first open bolls that opened the latter part of July. These bolls were collected from the field just before the first picking and placed on the soil surface in outside cages on November 15.

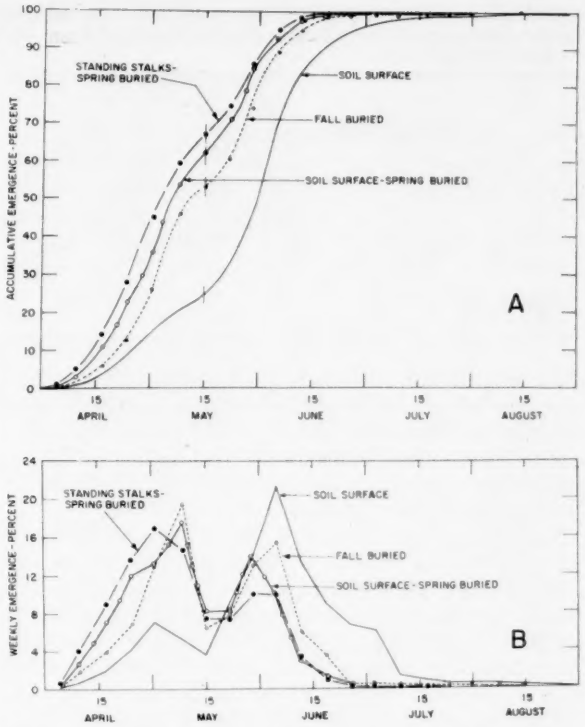

FIG. 1.—Time and rate of moth emergence of the pink bollworm from infested open cotton bolls in hibernation cages in four environments, 4-year average. *A*, accumulative emergence; *B*, weekly emergence.

Field observations showed that winter survival occurred in all infested fields, regardless of the date of stalk destruction or the type or date of cultural practices. They also showed a low winter survival when stalks were shredded and the land was plowed in the fall so that most of the crop residue was buried. A high survival was indicated when bolls remained on or near the soil surface throughout the fall and winter months. These data suggest the value of deep fall plowing for burial of the crop residue as a practical method of pink bollworm control in central Texas.

RELATION OF WEATHER TO SURVIVAL.—Weather is an important factor in limiting the carryover of the pest from one cotton crop to the next. Temperature, rainfall, and the average winter survival of the pink bollworm in the four environments are shown in table 3. In all environments except where the bolls were buried in November, winter survival was significantly higher in the two years of near normal rainfall than in the 2 drouth years. Mean and minimum temperatures were about the same

Table 2.—Winter survival of the pink bollworm in infested open bolls installed in outside cages in various environments.

Environment	Per Cent Survival					Equivalent Angle
	1952–53	1953–54	1954–55	1955–56	Average	
Buried November 15	1	2	4	1	2	7
Standing stalks, buried February 15	17	3	15	4	10	17
Soil surface, buried February 15	22	3	21	6	13	20
Soil surface, not buried	25	17	56	10	27	30
L.S.D. at 5% level	—	—	—	—	—	9

Table 3.—Winter survival of the pink bollworm in open cotton bolls in relation to temperature and rainfall from November through July.

Year	Temperature (°F.)		Rainfall (Inches)		Per Cent Survival	Equivalent Angle
	Mean	Minimum	Total	Departure from Normal		
1952–53	64.5	21	30.25	+ 3.93	16	22
1953–54	64.4	19	13.62	−12.69	6	13
1954–55	64.4	21	21.77	− 4.54	24	28
1955–56	64.7	20	11.21	−15.10	5	12
L.S.D. at 5% level	—	—	—	—	—	9

for each year. In this area of comparatively mild winter temperatures, the winter kill was approximately the same in bolls that were installed on the soil surface during the fall and winter months as in those on standing stalks during the same period. These data suggest that temperature was a minor factor affecting winter carryover.

Carryover of Larvae in Free Cocoons.—To determine if larvae overwinter in free cocoons in the soil, 4848 larvae collected from green bolls were installed in outside screened cages from September 13 to October 20, 1955. The following year 39 moths emerged—2 in April, 29 in May, 7 in June, and 1 in July. The winter survival was about 0.8%. Most of the larvae that cut out of green bolls in this area are of the short-cycle type, and consequently pupate and emerge within a short time. Winter carryover in this environment is considered of minor importance.

Survival in Blooms.—A total of 832 infested blooms were placed in outside cages on the soil surface between September 30 and October 26, 1955. Nineteen moths emerged from this material from March 29 to June 28, 1956, a winter survival of approximately 2.3%.

Duration of Resting Stage.—In central Texas resting larvae occurred every month of the year under field conditions. The maximum duration of the resting stage was about 10 months in open bolls on the soil surface and approximately 9 months in bolls buried 2 inches in the fall. These data are based on moth emergence records in hibernation cages during the 4-year period.

Larvae in Secondary Host Plants.—Infested fruiting forms of various wild and cultivated plants other than cotton were collected during the fall months and placed on the soil surface in outside cages to determine the degree of infestation and the time and rate of moth emergence.

It was found that the insect can maintain itself from one cotton crop to the next in the fruiting forms of the following malvaceous plants: *Hibiscus cannabinus* L., *H. coccineus* Walt., *H. esculentus* L., *H. lasiocarpus* Cav., *H. rosa-sinensis* L., *H. syriacus* L., *Malvaviscus drummondii* (T. & G.) Gray, *Althaea rosea* (L.) Cav., *Sida spinosa* L., and *Malachra capitata* L. Other host plants in this area included *Malva sylvestris* L., *Abutilon incanum* (Link) Sweet, and *Callirhoe lineariloba* (T. & G.) Gray, but winter carryover from these plants has not been established.

The most favorable alternate host plants in this area belong to the genus *Hibiscus*. The species of this genus that normally produce seed capsules become rather heavily infested when growing near heavily infested cotton. Fortunately, only two species, *syriacus* and *esculentus* (okra), are abundant enough to be of any importance. *Althaea rosea* and *Malvaviscus drummondii* usually become rather heavily infested under the same conditions, although these plants are scarce and of little importance.

The insect was found to breed on *Althaea rosea*, *Malva sylvestris*, and *Callirhoe lineariloba* in the spring before fruiting forms developed on cotton for pink bollworm propagation.

Summary.—Investigations were undertaken in 1952 at Waco, Tex., to determine the various factors influencing winter carryover of the pink bollworm (*Pectinophora gossypiella* (Saund.)) in central Texas.

Part of the larvae from each brood that developed in green bolls entered the resting stage. Very few overwintering larvae were present during July or August; their abundance rapidly increased during September, and by October 93% or more had entered this stage.

Most of the winter carryover was in the open bolls and locks of cotton left in the field. It was significantly higher in open bolls on the soil surface and lower in bolls buried in November than in any of the other treatments. There was no significant difference in winter survival between the two February-buried treatments.

Burial of infested open cotton bolls increased the rate of emergence, and consequently a larger percentage emerged from these environments before cultivated cotton was available for propagation.

Overwintering larvae occurred every month of the year under field conditions, and the maximum duration of the resting stage was about 10 months.

Winter survival also occurred from resting larvae in the shed blooms and from larvae in free cocoons in the soil, but these sources of winter carryover were considered of minor importance.

In all environments, except where the bolls were buried in November, winter survival was significantly higher in the 2 years of near normal rainfall than in the 2 drouth years. Mean and minimum temperatures appeared to be of minor importance.

It was found that the insect can maintain itself from one cotton crop to the next in the fruiting forms of at least 10 malvaceous plants, the most important of these being okra and *Hibiscus syriacus*.

The data suggest the value of deep fall plowing for burial of the crop residue as a practical method of pink bollworm control for central Texas.

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Control of the Clover Root Curculio on Alfalfa[1]

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The importance of the clover root curculio, *Sitona hispidula* (F.), in Virginia has been somewhat overshadowed by the recent occurrence of the alfalfa weevil, *Hypera postica* (Gyll.), and the spotted alfalfa aphid, *Pterocallidium* sp. While the damage caused by the larvae of the clover root curculio is not as conspicuous as that caused by insects feeding on alfalfa above ground, it has been observed (Underhill *et al.* 1955) that such damage causes considerable weakening of the plant and also that larvae feeding on the roots of alfalfa caused an increase in the number of plants heaved out of the ground by winter freezing and thawing. This damage has also been reported in other states by Webster (1915), Bigger (1930), and Marshall & Wilbur (1934).

In central Illinois, Bigger (1930) reported that as many as 43 larvae per square foot of soil could be found in infested areas. In Virginia, the writer has found as many as 22 larvae per square foot of soil in many alfalfa fields. In spite of these heavy infestations, the damage to the plants is not apparent until the roots of the infested plants are examined. The insidiousness of this damage has been pointed out by Jewett (1934), who reported that plants infested with the curculio for two seasons showed a reduction in hay yield of 18.6% over plants that were not infested.

Natural Control.—Although several parasites of the clover root curculio are known to exist in Europe, there are no known native parasites in this country as far as can be ascertained. In England, Jackson (1922) reported *Perilitus rutilus* Nees, *P. aethiops* Nees (= *Microctonus aethiops*) and *Pygostolus falcatus* Nees as parasitizing the curculio. In France, Berry & Parker (1950) reported that several species of *Sitona*, including the clover root curculio, were parasitized by *Campogaster exigua* (Meig.), in addition to the above species. They also reported that all the above-mentioned species of parasites are being imported into the United States in small numbers by the United States Department of Agriculture and the North Dakota Agricultural Experiment Station in a joint project.

A fungus, *Botrytis bassiana* (Balsamo) Montagne, was reported by Jackson (1922) as the most serious natural enemy of the clover root curculio in Europe. The organism attacked both larvae and adults. Rockwood (1951) discovered what he believed to be a pleophagus species of *Beauveria* in the Pacific Northwest attacking large numbers of the curculio. Wildermuth (1910) reported the larvae of this insect being attacked by a fungus of the Entomophthorae group.

During the summer of 1953, at Blacksburg, Virginia, a large number of newly emerged clover root curculio adults were attacked by a fungus. Samples of these adults were collected and sent to the Division of Mycology and Disease Survey, Agricultural Research Service, Beltsville, Maryland. The fungus attacking the adults was identified by Dr. Paul Lentz as *Beauveria globulifera* (Speg.) Picard. This fungus was reported earlier by Charles (1941) as attacking the clover root curculio in Virginia and Maryland. Judging from the numbers of dead adults that were observed being attacked, it is believed that this fungus may, in certain years, reduce the population of the curculio to the extent that it is not an economic problem on alfalfa.

Chemical Control Studies.—In the summer of 1953, preliminary yield data were taken on certain insecticide plots set up by G. W. Underhill, who has since retired from the Virginia Agricultural Experiment Station. The plots 20 by 30 feet in size, were laid out in a field known to be heavily infested with the clover root curculio. On April 11, 1953, the above-mentioned plots were treated with granulated dieldrin, BHC, aldrin, and toxaphene at the rates of 2, 2, 4, and 4 pounds per acre, respectively. The treatments were replicated two times.

On July 11, 1953, three random samples were taken from each plot on second-crop alfalfa with a quadrate that was 3 square feet in area. Thus the total sample for each plot represented a total area of 9 square feet. The samples of green weight hay were weighed and the weights of the treated plots were compared with those of the untreated plots.

It was found that plots treated with 2 pounds of actual dieldrin per acre showed an increase in weight of 44% over the untreated check plots. Plots treated with BHC, aldrin, and toxaphene showed less increase in yield over the untreated checks, but all of them gave some increase. Prior to the cutting of this alfalfa, there appeared to be only slight differences in the plots. On closer examination, the plants in the treated plots were seen to retain more of the lower leaves and to have thicker stems than those in the untreated plots. Also, there were fewer weeds in the treated plots, indicating that the stand of alfalfa was thicker. It was concluded that the application of certain soil insecticides could increase both the quality and quantity of alfalfa hay by protecting the roots of the plants from the larvae of the clover root curculio.

Residual Effectiveness of Several Soil Insecticides.—A test was set up in August 1953 to determine the length of residual effectiveness of three soil insecticides. Plots 30 by 50 feet in size were randomized and replicated four times in a field of newly seeded alfalfa. Dieldrin, aldrin, and heptachlor were applied at the rates of 2, 4, and 2 pounds of actual toxicant per acre, respectively. The numbers of applications are shown in table 1. Half of the treatments were applied in 1953 only, and the other half were applied in 1953 and every year thereafter through 1955.

For the treatments applied in 1953, aldrin and dieldrin were formulated on granulated tobacco stems at a strength of 2% active ingredient. Heptachlor was formulated on 30/60 mesh granulated attapulgite at a strength of 10% active ingredient. It was found later that the heptachlor formulation was deactivated by the attapulgite carrier. A sample of this material, analyzed by the Velsicol Chemical Corporation, was found to con-

[1] Accepted for publication April 12, 1957.
[2] The writer wishes to acknowledge the assistance of W. T. Keeton in the field experiments.

Table 1.—Control of the clover root curculio by the use of residual soil insecticides. Blacksburg, Virginia.

Insecticide	Pounds of Toxicant per Acre	Per Cent Reduction of Root Injury[a]		
		1954	1955	1956
Treatments Applied Only in 1953				
Dieldrin	2	75	72	64
Aldrin	4	60	50	35
Heptachlor	0.7[b]	44	31	25
Treatments Applied in 1953, 1954, and 1955				
Dieldrin	2	67	72	87
Aldrin	4	67	73	82
Heptachlor	2[b]	37	48	70

[a] Determined as described under "Residual Effectiveness of Several Soil Insecticides" in text.
[b] Analysis of the material revealed only 37% of its labeled strength; therefore only 0.7 pound instead of the intended 2 pounds was applied in 1953.

Table 2.—Control of the clover root curculio with soil insecticides applied in the spring or fall. Charlotte Court House, Virginia. 1954–55.

Treatments[a]	Pounds of Toxicant per Acre	Per Cent Reduction of Root Injury[b]	
		Fall[c]	Spring[d]
Captan (10%)	50	25	7
Isodrin (2%)	4	79	76
Toxaphene (10%)	20	79	78
Aldrin (2%)	8	89	79
Heptachlor (2%)	4	87	97
Dieldrin (2%)	4	97	91
Chlordane (5%)	20	95	97
Captan (10%) and Heptachlor (2%)	50 and 4	97	97

[a] All formulations of granulated tobacco stems except captan which was formulated as a dust.
[b] A vertical line connects the treatments that are not significantly different. A break in the line denotes significance at the 5% level (Duncan 1955).
[c] Applied September 7, 1954.
[d] Applied March 23, 1955.

tain only 37% of its labeled strength of heptachlor. Therefore, instead of the intended 2 pounds of actual heptachlor per acre being applied in the plots, only 0.7 pound was actually applied. For the applications made in August 1954 and September 1955, all of the insecticides were formulated on granulated tobacco stems at a strength of 2% active ingredient.

The applications for the 1953 and 1954 treatments were made by the use of a 24-inch Scott[3] fertilizer spreader. A 36-inch Gandy[4] fertilizer spreader was used for the application of the 1955 treatments.

Each summer, from 1954 through 1956, 20 plants were dug at random from each of the plots. Previously, it was determined that 20 plants per plot were an adequate size sample to use in all of these experiments. These plants were bound in bunches, labeled, and carried into the laboratory where they were washed and examined for root injury.

In order to determine the amount of root damage in the plots, a rating index system was used which converted the degree of root injury to a numerical figure. Numerical weights of 0, 1, 2, 3, and 4 were given to each root depending on the amount of root injury. The lower figures, such as 0, 1, and 2, were assigned to roots showing slight or no injury and the higher figures were assigned to the roots showing heavy or severe injury. The weighted total for 20 plants was calculated for each treated plot and the per cent reduction of root injury was determined by comparison with that in the check.

As indicated in table 1, all of the insecticides gave a substantial reduction in root injury when the treatments were repeated every year after the initial treatment. Heptachlor was somewhat less effective, probably due to the low dosage unintentionally applied in the initial application.

Of the treatments that were not repeated after the initial application, only dieldrin showed effective control of the clover root curculio after the first year. The residual effectiveness of this material appeared to have lasted for at least 3 years, although it had declined somewhat by the end of the third year. Such a residual effect could have immediate practical advantages. With the application of dieldrin at the time of alfalfa seeding, control of the clover root curculio could be obtained for at least 3 years without retreatment.

SPRING VERSUS FALL APPLICATIONS.—In previous work by Underhill *et al.* (1955) fall applications of several of the chlorinated hydrocarbon insecticides were somewhat more effective than spring applications for control of the clover root curculio. On the other hand, excellent control was obtained with heptachlor, which was applied only in the spring.

On a field of alfalfa seeded in February 1954, a test was designed to confirm the above work. Several insecticides, formulated on granulated tobacco stems were applied as shown in table 2. By the use of a split plot layout, half of the plots were treated on September 7, 1954, and the other half were treated on March 23, 1955. Application was made by the use of a 24-inch Scott fertilizer spreader. The plots, replicated three times, were 12 by 18 feet in size or approximately 1/200 acre.

Samples of 20 roots were taken from each plot in September 1955. Root injury was then evaluated by the rating index system previously described. The data were submitted to analysis of variance, after which significance at the 5% level was calculated by the multiple range test (Duncan 1955).

In all of the insecticide treatments, satisfactory control of the clover root curculio was obtained. As shown in table 2, there appeared to be little difference between the treatments applied in the fall and those applied in the spring. Analysis of variance indicated that no significant difference was obtained at the 5% level due to time of application. On the other hand, significance at the 5% level was obtained between treated and untreated plots and between certain insecticides. Heptachlor, chlordane, and dieldrin were slightly more effective than the other insecticides. Based on the results of this experiment, it is evident that good control can be obtained by applying any one of several chlorinated hydrocarbon insectides either in the early fall or early spring.

GRANULATED INSECTICIDE TESTS IN 1955.—Although successful control was obtained in the above experiment

[3] O. M. Scott and Sons Co., Marysville, Ohio.
[4] E. S. Gandrud Co. Inc., Owatonna, Minn.

by the application of heptachlor, dieldrin, or chlordane, the dosage rates were quite high. It was believed that granulated insecticides could be applied at lower dosages and still give good control. In September 1955, an experiment was laid out to compare the effectiveness of certain chlorinated hydrocarbon insecticides, at lower rates of application, with certain nematocides and phosphate insecticides. All of the materials at several strengths were formulated on different sized granules using attapulgite (table 3). The application of the materials was made immediately before the seeding of alfalfa, and they were mixed thoroughly into the top layer of the soil. A Gandy 36-inch fertilizer spreader, calibrated for each type of granulated material, was used in these applications. The plots were 24 by 44 feet in size, and they were replicated four times in randomized blocks. On September 8, 1956, the roots were dug from each plot and injury evaluated as previously described.

Heptachlor, alone or in combination with other materials, was particularly effective in reducing the amount of injury by the curculio (table 3). On the other hand, Nemagon, which is primarily a nematocide showed practically no control. Of the phosphate insecticides, Thimet showed very little control but parathion, applied at the rate of 5 pounds per acre, gave good control. Again most of the chlorinated hydrocarbon insecticides, with the exception of toxaphene and endrin, resulted in good to excellent control at these lower dosages. Dieldrin, at the rate of 4 pounds per acre showed no significant difference in control over this same material applied at the rate of 2 pounds per acre. Chlordane, at 5 pounds per acre also resulted in good control.

SOIL INSECTICIDES APPLIED AS SPRAYS.—In most of the previous research on the control of the clover root curculio, the insecticides were applied to the soil in granulated form by means of a fertilizer spreader. In August of 1955 an experiment was set up to determine the effectiveness of three insecticides applied as sprays for control of the clover root curculio. About 1 hour prior to alfalfa seeding, dieldrin, endrin, and haptachlor were each applied at the rate of 1 pound of actual toxicant per acre. The liquid spray was applied at the rate of 20 gallons per acre by means of a low pressure weed sprayer[5] mounted on a pickup truck. The plots were replicated six times. Two swaths 13 feet wide were made in each plot. The length of the plots varied, depending on the shape of the field, from 75 to 150 feet. Buffer zones, approximately 10 feet in width, were left between plots.

Table 3.—Control of the clover root curculio with granulated insecticides and nematocides. Chatham, Virginia. 1955.

TREATMENT	FORMULATION ON ATTAPULGITE	POUNDS OF TOXICANT PER ACRE	PER CENT REDUCTION OF ROOT INJURY[a]
Thimet (4%)	15/30	3	0
PCP[b]	—	2	0
Thimet (4%)	15/30	6	12
Endrin (1%)	30/40	2	23
Nemagon (5.1%)	15/30	17	31
Toxaphene (25%)	30/40	15	42
Dieldrin (5%)	30/50	2	62
Isodrin (5%)	30/50	2	64
Parathion (2½%)	30/50	5	67
Lindane (1%)	30/40	2	68
Chlordane (5%)	30/40	5	77
Dieldrin (5%)	30/50	4	78
Parathion (2½%)+ heptachlor (2½%)	30/50	5 and 2.5	87
Isodrin (5%)+ heptachlor (2½%)	30/50	2 and 2.5	96
Heptachlor (2½%)	30/50	2.5	99

[a] A break in the vertical line denotes significance at 5% level.
[b] Pentachlorophenol, (3 pounds per gallon) applied as a drench.

In November 1956, samples of the alfalfa roots from each plot were examined and evaluated for the degree of larval injury in the usual manner. Heptachlor and dieldrin, applied at the rate of 1 pound per acre, resulted in fairly effective control by reducing root injury 71 and 56%, respectively. Endrin gave practically no control, only 16% reduction in root injury. Analysis of the data showed that there was no difference at the 5% level between the dieldrin and heptachlor treatments. However, there was significant difference at the 5% level between these treatments and the endrin treatment.

Although the results in this test show that the percentage reduction of root injury was not as great as obtained with the granulated treatments, it should be pointed out that the dosage rates of the materials in the spray tests were lower than in any of the previous tests. It is believed that the results here would have been similar to those obtained in the previous experiment had the higher rates of application been used.

SUMMARY.—Although the occurrence of the alfalfa weevil, *Hypera postica* (Gyll.), and the spotted alfalfa aphid, *Pterocallidium* sp., on alfalfa in Virginia have overshadowed the importance of the clover root curculio, *Sitona hispidula* (F.), the root damage by this latter insect can cause a substantial reduction in hay yield.

During the summer of 1953, a fungus, *Beauveria globulifera* (Speg.) Picard, was found attacking newly emerged clover root curculio adults. It is believed that this fungus is an important natural enemy of the insect and responsible for reducing the population to the extent that it is not an economic problem in certain years.

In preliminary control studies with soil insecticides, the plots receiving dieldrin at the rate of 2 pounds per acre yielded an increase in green hay of 44% over untreated plots, presumably because of the protection given to the roots against the larvae of the clover root curculio.

An experiment to determine the residual effectiveness of dieldrin, aldrin, and heptachlor showed that dieldrin at the rate of 2 pounds of actual toxicant per acre gave protection against the curculio for 3 years. The other materials were effective for only 1 year.

Tests using insecticides in the granulated form showed that dieldrin, heptachlor, and chlordane, applied at the rates of 4, 4, and 20 pounds of actual toxicant per acre, respectively, resulted in excellent control when applied either in the early fall or early spring. A later test using these insecticides and several other granulated materials showed that the dosage rate of heptachlor, dieldrin, and chlordane could be reduced to 2.5, 2, and 5 pounds per acre without reducing the effectiveness of the treatments. Other materials such as Isodrin and parathion also gave

[5] The Engine Parts Manufacturing Co., 1360 West Ninth St., Cleveland 13, Ohio.

good control of the damage caused by the clover root curculio.

One pound of heptachlor or dieldrin, applied as a liquid spray to the soil at the time of seeding, resulted in fair control of the clover root curculio.

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The Effectiveness of Various Pesticides against the Cyclamen Mite on Strawberries[1]

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The cyclamen mite, *Steneotarsonemus pallidus* (Banks), is an important pest of many commercially grown plants. Some of the numerous ornamentals grown in the greenhouse which are attacked include African violets, begonias, chrysanthemums, cosmos, cyclamen, English ivy, fuchsias and larkspur. Although the cyclamen mite can not survive on most of these hosts when grown outdoors, it is an important pest of field grown strawberries. Some strawberries are moderately tolerant to cyclamen mite attacks, but others, particularly the everbearing varieties, are very seriously damaged. Since the introduction of several highly susceptible varieties into California, the cyclamen mite has become the most destructive pest of strawberries in that state.

The cyclamen mite, which feeds on the new tender growth of plants, secretes a toxin which causes severe stunting and distortion of infested plants. This curled and twisted growth not only provides a highly suitable habitat for the mites, but also makes it very difficult to contact them with a pesticide. Since the mites prefer new growth, they are to a certain extent protected from residual deposits of pesticides, for they soon move to new clean growth which is produced after pesticides are applied.

Since its description by Banks (1901) the cyclamen mite has been notorious because of difficulty encountered in attempts at control. Not only are the mites difficult to contact with a spray or dust, but they are highly resistant to most pesticides. In addition, the cyclamen mite has such a high biotic potential that it is essential to obtain very high mortality if control is to be obtained for a practical length of time. Under certain circumstances moderate control might make it possible for a predator to hold the population at a low level; however, this would only happen when there was a relatively high predator population, and the pesticide was innocuous to the predator. On strawberries control is further complicated by the residue problem. For example, under California conditions strawberries are picked weekly from April until November and interruption of picking for even 1 week can result in the loss of several hundred dollars per acre.

Early research workers (Britton *et al.* 1915, Compton *et al.* 1930, Goodhue & Smith 1944; Greenslade 1935, Massee 1933, Munger 1933, Smith 1935) reported varying degrees of control of the cyclamen mite with naphthalene, paradichlorobenzene, nicotine, lime sulfur, lauryl thiocyanate and sulfur; however, since none of these materials gave practical control, the only recommended treatment was a hot water dip at 110° F. for 30 minutes (Massee 1933, Smith & Goldsmith 1936). Reports on the effectiveness of the new organic insecticides commenced in 1946 when Massee stated that both DDT and BHC were ineffective. Haight (1949) and Montgomery (1955) reported that sodium selenate would give control on ornamentals. Pritchard & Beer (1949) reported favorable results with toxaphene when it was used repeatedly, and subsequently, (Pritchard 1951) mentioned that satisfactory control could be obtained with parathion sprays if the mites were unprotected as they occur on certain ornamentals. Neiswander (1952) stated that Genite dust would give control on azaleas. Hahman & Müller (1951, 1952) working on strawberries in Germany showed that demeton and DDT had little effect on the cyclamen mite, whereas parathion and lindane, when used repeatedly, would give marked reductions of field populations. Van den Bruel & Colin (1953), and van den Bruel & Derard (1953) reported parathion more effective than lindane, but even though parathion could cause marked reductions of cyclamen mite populations, they soon returned to

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previous levels when treatment was discontinued. Subsequently van den Bruel (1954) reported that aldrin, Aramite, demeton, dieldrin, Dimite, EPN, lime sulfur, malathion, and schradan were all ineffective, and that the only practical control on strawberries was to plant non-infested nursery stock. More recently Allen (1955) and Morishita & Jefferson (1955), working with strawberries and ornamentals, respectively, reported that endrin was a highly effective material against the cyclamen mite. Recently Kelthane has been reported as an effective control by Jefferson & Morishita (1956), Neiswander & Moesel (1956), and Hodson & Cutkamp (1956).

The present investigation was initiated in 1952 with the objective of assembling quantitative data on cyclamen mite control which might indicate certain groups of pesticides which would be most effective against this mite. Most of the early reports merely mentioned that good or poor control was obtained, but little comparison could be made between reports. This paucity of data is understandable, for it is very difficult to make counts of the cyclamen mite because of its small size and tendency to remain concealed.

METHODS.—These experiments were conducted in heavily infested strawberry fields which were selected for their comparatively even cyclamen mite populations. Shasta, Lassen and 20th Century varieties were used in these experiments, but only one variety was used for any one test. The small plots, each covering 9 feet of double-rowed bed, were arranged in randomized blocks; there were two replications for each treatment. The sprays were applied with a hand sprayer at the rate of $\frac{1}{2}$ gallon per plot which is equivalent to 700 to 800 gallons per acre depending on the width of the furrows. These high gallonages were used with more than adequate dosages with the intent of quickly eliminating those materials which did not give a high degree of control. In all the tests additional wetting agent ($\frac{1}{2}$ pint per 100 gallons) was added to insure maximum penetration of the spray into the folded leaves and crowns of the plants. Pre- and post-treatment counts were made by picking 15 (11 in the first experiment) folded leaves from each replication and counting the number of individuals found within a 7-mm. circle placed over the base of the center leaflet. Separate counts were made of the adults, immatures and eggs. In order to prevent undue emphasis on any one sample, counts were restricted to 100 individuals for each stage on any one leaflet. Since there were no differences in mortality between the stages the summarized counts represent a total count for all stages. This method of tabulation gives the most critical evaluation of materials, for the eggs greatly outnumber all the other stages.

The population declines that were occasionally encountered in the untreated checks were caused by several factors. Often the mites were so abundant that the strawberry plants could not continue to support such high populations. In addition, leaf samples which were taken repeatedly from each plot removed a detectable portion of the population; however, this was considered less detrimental than increasing the plot size, and consequently, increasing the population variation within each plot. Since it was known that the predatory mites *Typhlodromus cucumeris* Oudemans and *T. reticulatus* Oudemans could effectively reduce cyclamen mite populations (Huffaker & Kennett 1953, 1956), a complete count of the predators was made in each experiment.

The treatments and results of these experiments are summarized in table 1. Materials which did not give a high degree of control were rejected after a single test. Materials which showed promise were always tested a second time, and in the later experiments endrin was used as a standard treatment for comparison.

RESULTS.—In experiment 1, azobenzene at 2 pounds actual per 100 gallons, although slow in its action, reduced the population 99.6% by the second week and held it at this level for 5 weeks. Counts which were continued for longer than shown in the table indicated that control was still evident after 10 weeks. Although parathion at 0.9 pounds actual per 100 gallons gave the highest initial mortality (96.2%), subsequent counts revealed that the mites increased rapidly after the first week. Separate counts of the adults, nymphs and eggs indicated that this was not due to differential tolerances among the developmental stages. Lindane at 0.4 pounds actual was similar in its action to parathion although it seemed to have a slightly longer residual action. Dieldrin at 0.3 pounds actual, although not highly effective, did give sufficient control in the second and third weeks to suggest that widely different materials should be tested against the cyclamen mite. The acaracides Aramite, Chlorobenzilate and Dimite were all relatively ineffective against the cyclamen mite. Shell OS-1836, a short residual systemic, produced 80.7% control which is considerably better than that obtained with any of the other systemics; however, it seems likely that this material was acting as a non-systemic phosphate. A second application a week after the first did not enhance control appreciably which suggests that the material did not reach some of the mites in protected niches. Calcium polysulfide and rotenone both of which had been suggested for control in the early literature gave only a small degree of control.

In experiment 2, endrin at 0.4 pounds actual per 100 gallons gave 95 to 99% control for the first 5 weeks, and additional counts, not shown in the table, disclosed that control persisted for twice this long. Isodrin at the same dosage was less effective than endrin throughout the period in which counts were made. Parathion gave results similar to those in experiment 1. Methyl parathion, at 0.5 pound actual was much less effective than parathion at 1 pound and 0.5 pound in experiment 3. Both heptachlor and aldrin caused some mite mortality, but they were certainly not effective materials. Ethylene dibromide when applied emulsified in water gave negligible control, but ethylene dibromide stimulated sufficient plant growth to falsely suggest mite control.

In experiment 3, endrin once again gave the best control and isodrin was clearly less effective which was true in all of the tests. Lindane and parathion, when used at 0.4 and 0.5 pound actual, respectively, proved to be about equally effective. Dieldrin at 0.3 pound actual and aldrin and heptachlor at 0.5 pound seemed to be more effective than chlordane at 0.5 pound, but as previously shown they gave only partial control.

In experiment 4, cool weather and some rain followed the spray application. Even though the cool weather and poor condition of the plants caused a marked decline in

Table 1.—The effectiveness of various pesticides applied as sprays for control of the cyclamen mite on strawberries in California. 1952–56.

Material	Pound of Active Ingredient per 100 Gal.[a]	Pretreatment Count[b]	Per Cent Reduction Under the Pre-Treatment Count on the Indicated Days After Application				
			7	14	21	28	35
Experiment 1—San Jose, July, 1952							
Azobenzene	2.0	542	73.6	99.6	99.6	—	99.6
Dieldrin	0.3	705	48.8	87.7	94.2	—	77.4
Lindane	0.4	754	78.4	87.9	79.4	—	57.4
Parathion	0.9	547	96.2	84.5	69.7	—	78.2
Shell OS 1836	0.5	565	80.7	71.9	36.3	—	—
Calcium polysulfide	0.5	675	76.0	61.5	9.8	—	—
Dimite	0.5	613	57.6	67.4	66.1	—	+12.2
Chlorobenzilate	0.5	702	43.2	64.1	74.9	—	30.2
Toxaphene	1.4	489	42.9	65.0	5.5	—	—
Aramite	0.5	925	68.6	54.5	34.2	—	—
Rotenone	0.1	750	30.3	45.5	39.2	—	—
Untreated	—	802	6.9	2.6	40.4	—	+33.5
Experiment 2—San Jose, April, 1953							
Endrin	0.4	812	99.3	94.7	96.4	—	97.3
Isodrin	0.4	1015	78.6	90.8	92.2	—	82.8
Parathion	1.0	759	90.5	75.6	77.5	—	82.1
Heptachlor	0.5	568	79.0	88.0	14.3	—	38.2
Aldrin	0.5	968	72.9	49.4	43.8	—	48.7
Methyl parathion	0.5	609	7.9	+34.2	—	—	—
Ethylene dibromide	2.5	669	20.6	+13.2	—	—	—
Ethylene chlorobromide	2.1	1553	+10.6	+20.9	—	—	—
Untreated—	—	912	+75.3	+131.1	+115.9	—	92.1
Experiment 3—San Jose, May, 1953							
Endrin	0.4	2570	87.2	97.3	93.1	—	88.0
Isodrin	0.4	2548	70.3	88.7	77.6	—	60.5
Lindane	0.4	2848	77.7	71.2	55.8	—	28.2
Parathion	0.5	2304	56.5	67.4	21.6	—	—
Dieldrin	0.3	2032	37.6	52.8	40.3	—	+6.6
Aldrin	0.5	2718	54.5	48.2	32.4	—	—
Heptachlor	0.5	2551	53.7	40.0	4.9	—	—
Chlordane	0.5	2188	9.9	22.0	+2.1	—	—
Untreated—	—	2603	+24.2	+22.4	9.0	—	14.0
Experiment 4—Concord, June, 1954							
Endrin	0.4	993	57.0	94.4	98.4	99.3	—
Chlorbenside	0.8	639	26.8	68.4	76.1	47.9	—
Holcomb 326	0.5	665	37.4	52.6	41.8	62.4	—
Diazinon	0.5	548	50.2	42.2	21.4	27.7	—
Ovex	1.0	778	+5.4	28.4	52.7	16.6	—
Schradan	1.4	694	18.7	23.8	30.5	53.2	—
Demeton	0.5	743	+21.7	15.1	+19.7	39.7	—
TEPP	0.4	797	4.6	9.4	44.0	24.1	—
Pyrazoxon	0.5	677	+19.9	+4.3	17.0	36.3	—
Untreated	—	368	+61.7	47.0	59.2	81.0	—
Experiment 5—San Jose, July, 1954							
Endrin	0.4	3436	99.7	99.5	99.8	—	94.2
Azobenzene	4.0	2854	92.7	98.6	99.5	—	96.8
Isodrin	0.4	3071	96.6	97.5	95.8	—	86.6
Diazinon	0.5	3219	98.9	97.7	85.7	—	64.5
Strobane	1.5	2440	88.0	77.3	82.7	—	+9.1
Am. Cyanamid 12009	0.5	2580	81.9	66.0	49.6	—	—
Am. Cyanamid 12008	0.5	1916	72.5	57.1	+1.9	—	—
Untreated	—	2352	41.9	40.4	55.7	—	40.6

[a] Spray applied at 800 gallons per acre.
[b] Based on the number of mites inside a 7-mm. circle on 30 leaflets.

Table 1—(continued)

Material	Pounds of Active Ingredient per 100 Gal.[a]	Pre-Treatment Count[b]	Per Cent Reduction Under the Pre-Treatment Count on the Indicated Days After Application				
			7	14	21	28	35
Experiment 6—Watsonville, July, 1955							
Endrin	0.4	2319	86.4	96.1	91.6	—	—
Kelthane	0.4	2111	30.6	58.2	62.1	—	—
Phostex	0.8	2494	32.4	58.1	44.4	—	—
Thimet	1.0	2161	31.6	45.1	53.2	—	—
Chlorthion	1.0	2240	52.1	27.4	42.2	—	—
Trithion	1.0	2402	20.4	40.7	38.4	—	—
Am. Cyanamid 4124	0.5	1579	35.1	31.2	17.5	—	—
Guthion	0.5	2452	28.7	36.8	7.5	—	—
Hercules AC-528	0.5	2134	+7.1	41.4	24.2	—	—
Dipterex	1.0	2493	12.6	32.9	22.6	—	—
Untreated	—	1619	+24.5	+20.4	+26.4	—	—
Experiment 7—San Jose, July, 1956							
Endrin	0.4	1234	—	99.3	98.4	99.2	—
Thiodan	0.5	1642	—	98.9	95.6	99.1	—
Sevin	0.5	3133	—	66.0	72.6	81.2	—
Tedion	0.25	2274	—	+15.3	31.8	63.7	—
Chipman R-6199	0.5	1972	—	+7.5	38.1	44.5	
Bayer 19639	0.5	1362	—	+29.3	+87.2	+42.2	—
Untreated	—	3037	—	21.8	54.8	59.6	—

the untreated check, endrin, which was used as a standard treatment, was the only material which gave satisfactory control. Chlorbenside, Holcomb 326[2] and Diazinon gave some control, but they were not very effective. The poor performance of Diazinon compared with subsequent tests probably resulted from reduced fumigant action in the cool, wet weather. The three systemic pesticides schradan at 1.4 pounds, demeton and Pyrazoxon[3] at 0.5 pound were equally ineffective. Ovex and TEPP, like the other acaracides, gave negligible control.

In experiment 5, azobenzene at 4.0 pounds actual gave control comparable to endrin at one-tenth this dosage. Both held the population at a low level for 5 weeks, and after 7 weeks these two treatments were still better than the untreated check. These differences would have been larger if the small plots had not allowed a certain amount of reinfestation. Diazinon gave good initial control, but like parathion did not continue to reduce the population after the first week, and consequently permitted the population to soon revert to its previous level. Strobane resulted in only moderate mortality, and the two systemics, Am. Cyanamid 12008 and 12009, although used at the high rate of 4 pounds actual per acre, resulted in very little control.

In experiment 6 endrin was again far superior to any of the other materials tested. Kelthane at 0.4 pound actual per 100 gallons caused a 62.1% reduction after 3 weeks. In another test not shown in the table Kelthane at twice the dosage, 0.8 pound actual, gave approximately 80% control which was still inferior to the 99% control obtained with endrin in the same test. From these results it appears that the good control reported by Jefferson & Morishita (1956), and Neiswander & Moesel (1956) was dependent on repeated applications of Kelthane. The two phosphates Guthion and Thimet when applied at 0.5 pound actual and 1.0 pound actual, respectively, gave very poor control. Other materials which caused little reduction in the population were Phostex, Chlorthion, Trithion, Am. Cyanamid 4124, Hercules AC 528 and Dipterex.

Experiment 7 was an example of a plot in which the predator *Typhlodromus* had a marked effect on the populations. Nevertheless, it is possible to determine the effectiveness of most of the materials. Thiodan, a material related to endrin, gave comparable control to endrin when used at 0.5 pound actual per 100 gallons. Further tests will be necessary to determine whether it will be this effective under all conditions. Sevin[4] and Tedion seemed to be only moderately effective when one takes into account the drop in the check and the number of predators found in the plots. Both Chipman R-6199 and Bayer 19639 gave little or no control. Since they caused complete mortality of the predators, cyclamen mite populations in these plots did not decline to the extent that they did in the check.

Other experiments which are not represented in the table gave sufficient results to demonstrate that several materials did not warrant further testing. DDT and Dilan at 0.5 pound actual per 100 gallons were ineffective as was DDVP at 1.0 pound actual. DN-111 (dicyclohexylammonium dinitro-o-cyclohexylphenate) and DN-289 (triethyl anolamine salt of 4, 6-dinitro-2-sec-butylphenol) at 0.3 and 0.8 pound actual both gave partial control, but they caused severe plant injury Malathion at 1.0 pound actual had little effect as was true of Genite and Phosdrin at 0.5 pound. Other materials which had

[2] A product no longer made having the definition, 0,0-diisopropylphosphordithioic acid ester of N,N-diethyldithiocarbamic anhydrosulfide.

[3] Diethyl 5-(3-methylpyrazolyl) phosphate.

[4] N-methyl-1-naphthyl carbamate. Has also been referred to as C & C 7744.

little or no effect were lauryl thiocyanate, Neotran, nicotine, NPD (tetrapropyl dithiopyrophosphate) Perthane and pyrethrin.

DISCUSSION.—Dusts and low-gallonage sprays will probably never be effective against the cyclamen mite because of the difficulty of contacting the mites. Even when an effective systemic pesticide is found it will probably be necessary to use comparatively high-gallonage sprays, for generally there is little translocation of pesticides from the leaves down to the base of the plant. Although repeated applications will enhance control by allowing the plants to grow out of their stunted condition, it is of much greater importance that a sufficient gallonage be used with each application so that there is maximum penetration of the spray into the crown of the plant. The tetranychid mites, which have a highly resistant egg stage, can often be much more effectively controlled with several properly timed applications, however in the case of the cyclamen mite there does not appear to be any one stage which is more resistant to chemicals.

It is obvious that the cyclamen mite is very different from the tetranychid mites on which many conclusions concerning mite control are based. The acaracides such as Aramite, Chlorobenzilate, Genite, and ovex, are all ineffective against the cyclamen mite. On the other hand materials not usually thought of as acaracides such as endrin, isodrin and Thiodan give much better control.

The systemic pesticides, which would seem to be the best suited for controlling mites in inaccessible places, are in general ineffective against the cyclamen mite. Sodium selenate, one systemic which has been reported to give control, was not tried because of the impracticality of using a material which would persist in the fruit for a long period of time. The non-systemic phosphates vary considerably in their effectiveness with Diazinon and parathion being the most effective. Initial mortality is comparatively high with these two materials but control is not sufficient to prevent populations from rapidly returning to their former levels. Lindane, like parathion, has a very short residual effect. In these tests they were about equally effective, but at lower gallonages parathion might be superior because of its fumigant action

SUMMARY.—Spray applications of more than 50 pesticides were made to field grown strawberries to determine their effectiveness against the cyclamen mite. In these tests azobenzene, endrin and Thiodan proved to be the most effective materials. The commonly used acaracides and systemic pesticides were all ineffective. The phosphate pesticides varied greatly in their effectiveness, but even the best gave insufficient control to prevent rapid return of populations to their previous levels.

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The Effect of Conditioned Flour on the Toxicity of Sodium Fluoride to *Tribolium confusum* Duv.[1]

TAGE S. K. JOHANSSON[2] and MILDRED P. JOHANSSON[3]

Allee *et al.* (1949) have defined environmental conditioning as "a modification of the effective environment by population-group activities" and have reviewed research in this area. Park (1934, 1935, 1936, 1937) found that flour in which a population of the confused flour beetle (*Tribolium confusum* Duv.) lives and feeds becomes biologically altered and described such flour as "conditioned." He investigated primarily the effects of heavily conditioned flour and its dilutions on the population physiology of subsequent beetle cultures. The effects of conditioning may be due in part to the quinones which impart a characteristic pink color to such flour and are secreted by the abdominal and thoracic glands of adults (Roth & Howland 1941, Roth 1943, Alexander & Barton 1943). More recently, Loconti & Roth (1953) concluded that this material is not a factor in population growth of *Tribolium castaneum*. Although heavy conditioning is a deterrent to population growth, Park (1937) suggests that in certain concentrations, conditioned flour may have a stimulatory effect.

It has been demonstrated among other animal species that environmental conditioning can have a protective effect against toxic substances (Allee *et al.*). Such protection in *Tribolium* populations has not been investigated but Bissell (1943) found flour beetles surviving and reproducing in a 1:25 pound cryolite-corn meal poison bait over a period of 2½ years and Gough (1939) suggested the ethylquinone secreted may react with hydrogen cyanide used as a fumigant.

The following experiments were initiated to determine whether conditioning, well below the asymptotic population level, might be a factor in the response of the confused flour beetle to low levels of poisoning. Sodium fluoride was chosen as a stomach poison and mixed with the flour used to feed the beetles. A preliminary experiment using four replicates of 100 adult beetles, less than 6 months old, was run in dilutions of heavily conditioned, poisoned flour. A second series was tried using flour which had been inhabited by a specific number of beetles per gram for 2 and 3 weeks, respectively. Both sets of experiments showed a consistent lag in mortality of beetles living in some levels of conditioned compared with fresh, poisoned flour (Johansson 1947).

METHOD.—To establish reproducible levels of conditioning, the test flour was inhabited for 3 weeks by 1 to 12 beetles per gram, depending on the level of conditioning desired. The following tests using 50 males and 50 females per replicate were run on each of five levels of conditioned flour:

A. Poisoned with 0.5% sodium fluoride by weight (400 beetles per level)
   1. *single* beetles in individual vials with 0.5 grams of medium
   2. *pairs* of beetles of opposite sexes in individual vials with 1 gram of medium.
   3. pairs of beetles of the same sex (*doubles*) in individual vials with 1 gram of medium
   4. *groups* of 5 pairs of beetles of opposite sexes in individual vials with 2½ grams of medium
B. Poisoned with 1% sodium fluoride (200 *single* beetles per level)

Flour was conditioned by adding 150, 600, 1,200 or 1,800 beetles to 150 grams of flour (1, 4, 8 or 12 beetles per gram) and leaving in a constant temperature chamber for 3 weeks. At the end of this period, the flour was sifted to remove beetles, larvae, pupae and eggs. Flour so conditioned was then thoroughly mixed with NaF (0.5 or 1%) in a ball mill.

Test beetles (stock obtained from Dr Thomas Park, Dept. of Zoology, University of Chicago) were cultured in half-pint milk bottles containing 50 grams of unbleached flour (GLF). One hundred adult beetles were added to lay eggs in these stock bottles and removed after 1 week. Upon pupation, offspring were removed and sexed (Chapman 1918). Within 24 hours of emergence, adults were placed in shell vials containing ½ gram of medium per beetle.

Vials and stock cultures were held in a constant temperature chamber at 25° C. Beetles were checked daily and considered dead when they no longer moved in response to touch. Beetles apparently dead were left undisturbed for ½ hour to detect those feigning death. The number of beetles in the vials of pairs and groups was kept constant by replacing those that died with others of the same age, sex and stock. Colored finger nail polish was applied to the elytra to distinguish: a) males from females and b) replacements for dead individuals.

RESULTS AND DISCUSSION.—Analysis of variance showed a significant difference in 50% mortality of beetles inhabiting conditioned versus fresh flour poisoned with 0.5% sodium fluoride (table 1). Comparison of the results at the levels of conditioning used in this test indicated a peak effect with 8 per gram conditioned flour (figure 1). Analysis of the tests using 1% sodium fluoride (table 2) again showed some protection from conditioned compared with fresh flour. At this higher level of poisoning there was no detectable difference between 1, 4, 8 and 12 per gram conditioning. The mortality of beetles living in the five levels of unpoisoned, conditioned flour was 0.6% in 40 days.

The media were not changed during the above tests but as the ratio of one beetle to ½ gram of flour was kept constant, the conditioning effect of each beetle on its medium was constant. Park & Woollcott (1937) found that beetles living in the same medium for 40 days produced some conditioning of that medium. This additional conditioning effect is apparent (fig. 2) in the divergence of the longer lived beetles in 1 per gram and 4 per gram flour from the 0 per gram curve. Conversely, the 12 per gram beetles show the detrimental effects of high conditioning

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Table 1.—Fifty per cent mortality expressed in days of *Tribolium confusum* living in five levels of conditioned flour poisoned with 0.5% sodium fluoride.

Conditioning (No./Gram)	Singles		Pairs		Doubles		Groups	
	Male	Female	Male	Female	Male	Female	Male	Female
0	24.3	25.0	26.0	22.6	23.4	22.8	22.3	20.0
1	25.0	25.7	28.5	26.7	29.0	28.3	21.0	22.5
4	25.0	25.2	26.4	25.6	22.7	27.0	24.0	22.0
8	30.0	31.2	31.0	27.6	29.0	31.3	30.0	29.0
12	28.7	30.6	29.3	27.9	27.3	29.0	28.5	29.2

Sources of Variation	D.F.	S.S.	M.S.	F
Sampling	(7)	(53.450)	(7.637)	(2.917)*
male vs. female	1	.441	.441	.168
pairs (P) vs. doubles (D)	1	.162	.162	.061
singles (S) vs. P and D	1	.267	.267	.101
groups (G) vs. S, P and D	1	34.776	34.776	13.283**
sex vs. P and D	1	15.842	15.842	6.051*
sex vs. S, P and D	1	1.411	1.411	.538
sex vs. S, P, D and G	1	.560	.560	.213
Conditioning	(4)	(247.966)	(61.992)	(23.679)**
0 per gm. vs. conditioning	1	100.172	100.172	38.262**
linear	1	87.912	87.912	33.579**
quadratic	1	.101	.101	.038
cubic	1	59.780	59.780	22.834**
Error	28	73.306	2.618	

* Probability between 0.05 and 0.01.
** Probability less than 0.01.

by the increased mortality rate beyond the 25th day.

Although the amount of food and poison available to each beetle was constant, the mortality rate for beetles confined in groups of 10 was significantly higher than that for one or two beetles in a vial (table 1). Daily mortality checks of the groups necessitated emptying each vial to locate the 10 beetles whereas vials containing one or two beetles could be gently rotated to obtain the same information. The higher mortality in the groups may well be associated with greater activity induced by this daily disturbance. Gough concludes that shaking adult beetles is one of the factors in his mortality tests of beetles exposed to a fumigant.

Table 2.—Fifty per cent mortality expressed in days of *Tribolium confusum* living in five levels of conditioned flour poisoned with 1% sodium fluoride.

Conditioning (No./Gram)	Singles (Test 1)		Singles (Test 2)	
	Male	Female	Male	Female
0	19.9	18.9	19.6	20.2
1	21.9	21.1	20.8	21.9
4	20.2	21.1	21.1	22.2
8	21.3	20.4	21.5	21.7
12	21.4	19.7	22.2	22.1

Check (0 per gram) vs. conditioning: F = 24.773**
Among levels of conditioning: F = .149

Sex appeared to be a factor only when males and females were together. Comparing the mortality of beetles confined in vials containing two beetles of the same sex (doubles) with that for two beetles of opposite sexes (pairs) showed the males surviving longer than females

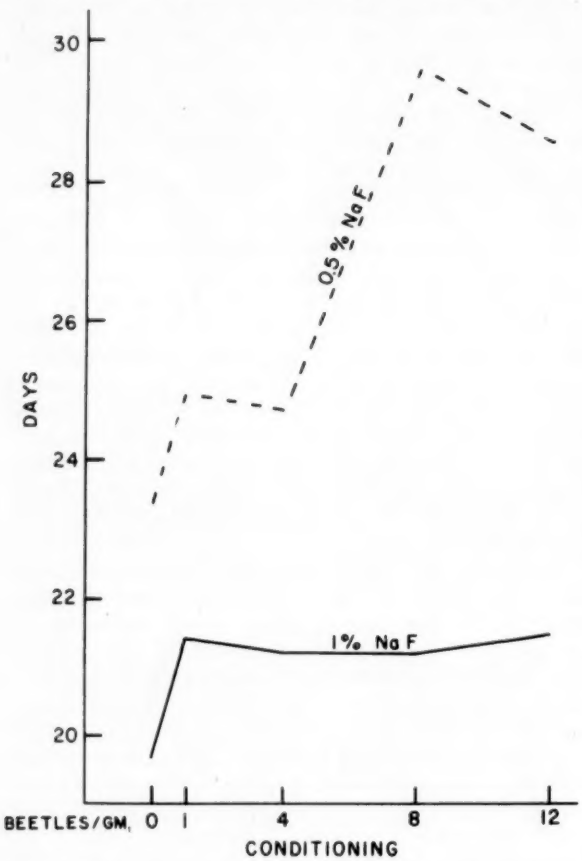

FIG. 1.—Fifty per cent mortality of *Tribolium confusum* living in five levels of conditioned flour poisoned with 0.5% and 1% sodium fluoride.

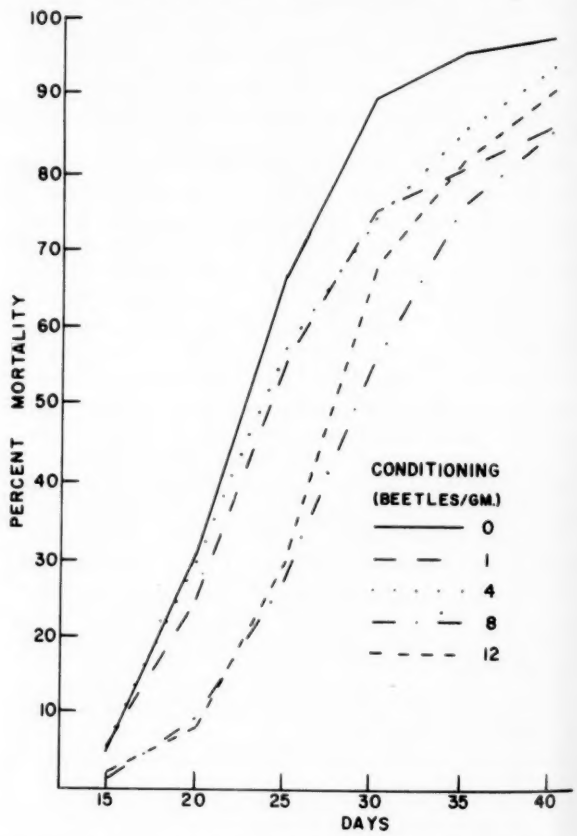

FIG. 2.—Per cent mortality of *Tribolium confusum* living in five levels of conditioned flour poisoned with 0.5% sodium fluoride.

only in the paired category. Gough, and Loschiavo (1955), present data comparing mortality of 0 to 1 day old males and females exposed to hydrogen cyanide and p-p′-DDT respectively. In both cases, there was no sex difference at this age but no comparisons of female mortality were made with and without males present.

SUMMARY.—Reproducible levels of conditioned flour were used to test their effectiveness in protecting *Tribolium confusum* against 0.5 and 1% sodium fluoride poisoning. Adults were exposed to poison within 24 hours of emergence. A lag in mortality was apparent with conditioned flour although eventually all beetles died in poisoned media. With 0.5% sodium fluoride, a decrease in protection was observed in conditioning beyond the eight beetle per gram level. The levels of conditioning tested showed no difference in protection of beetles against 1% sodium fluoride.

With a constant ratio of one beetle per ½ gram of medium, the mortality rate of beetles confined in groups of 10 was significantly higher as compared with singles, pairs or doubles. There was no over-all sex difference in mortality rate except that females paired with males, rather than another female, showed a higher death rate.

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An Electrical Device for Periodical Recovery of Pollen Collected by the Honey Bee[1]

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The main purpose for constructing this device was to collect automatically the pollen gathered by the honey bee, *Apis mellifera* L., at 2-hour intervals during the daytime. It was used successfully in the Kansas State College apiary during the active season of honey bees for the years 1955 and 1956.

DESCRIPTION OF POLLEN COLLECTOR.—This device is composed of a large wooden turntable (A) (Plate 1, fig. Z) 23 inches in diameter, divided into eight sections placed immediately below the pollen trap. Each section carries a metal pan (A^1) which remains stationary under the hardware cloth (the pollen load shearing device) of the pollen trap for the desired period. The motor assembly unit (B) (Plate 1, fig. X) is a 1/20 h.p. Dayton motor, 115 v., a.c., 6000 r.p.m., attached to a war surplus Signal Corps aerial reel wind gear assembly. Transmission of power is accomplished by a power take-off, consisting of a 2-inch V-type pulley that drives an 8-inch, step-down pulley (C) (Plate 1, fig. X). Attached to the bottom of the turntable is a 14-inch step-down pulley that is driven by the 2-inch pulley on pulley (C). Inserted in the electric circuit to the motor is a 60-watt light bulb acting as resistance to decrease the speed of the motor (Z) (Text figure 1). The timing mechanism consists of a metermatic time switch (Y) (Plate 1, fig. Y and Text fig.

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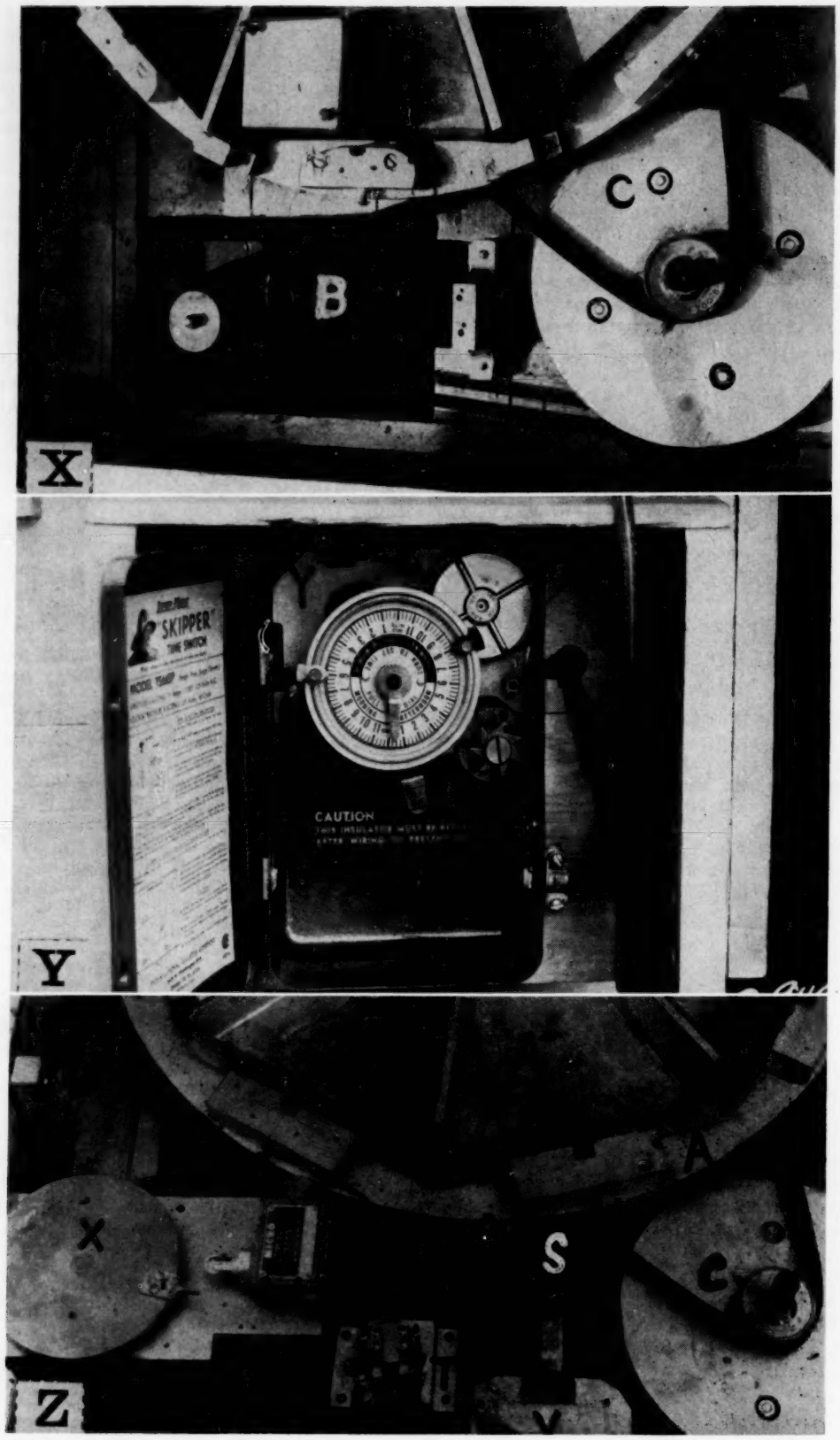


PLATE 1:

FIG. X.—Motor assembly unit, turntable and pulleys.

FIG. Y.—Metermatic time switch.

FIG. Z.—Motor assembly unit control: X, Haydon timer; W, microswitch; T, magnetic relay; V, relay tube, normally closed; S, microswitch, R, toggle switch.

1) which is enclosed in a small metal box, attached to the box enclosing the turntable near the motor. This time switch operates on the principle of an electric clock. The dial-disk, which rotates, is divided into hours while the time indicator is stationary. Attached to the dial-disk are two clips which can be fixed at the desired time limits of operations. At the desired time the clip fixed at the early hour of the morning (6 A.M.) touches a switch and completes the electric circuit. The Haydon timer (X) (Plate 1, fig. Z) makes one complete revolution every 2 hours. The contact pointer attached to the disk of the Haydon timer touches the microswitch (W) and closes the circuit which is normally open. When the pointer touches the microswitch, the electric circuit activates the magnetic relay (T) which limits the action of the turntable to one-eighth of a revolution.

Since it is essential that only one pan be moved when the motor rotates the turntable, two relay sets were inserted in the circuit. The tube relay (V) (Plate 1, fig. Z) is an Amperite relay tube (115 v., C3) which is normally closed. When the electric circuit is completed through the microswitch (W), the filament heats and opens the circuit for 2 seconds. The magnetic relay (T) (Plate 1, fig. Z) activates the tube relay (V) and also the motor assembly unit (B) (Plate 1, fig. X). The motor assembly unit is located beneath Haydon timer (X), magnetic relay (T), and microswitch (S). In 3 seconds the relay tube (V) changes the path of the current to the motor assembly through microswitch (S) which is normally closed. When the turntable has rotated one-eighth revolution, it opens microswitch (S) opening the circuit which stops the motor. Because the pans need to be emptied after the

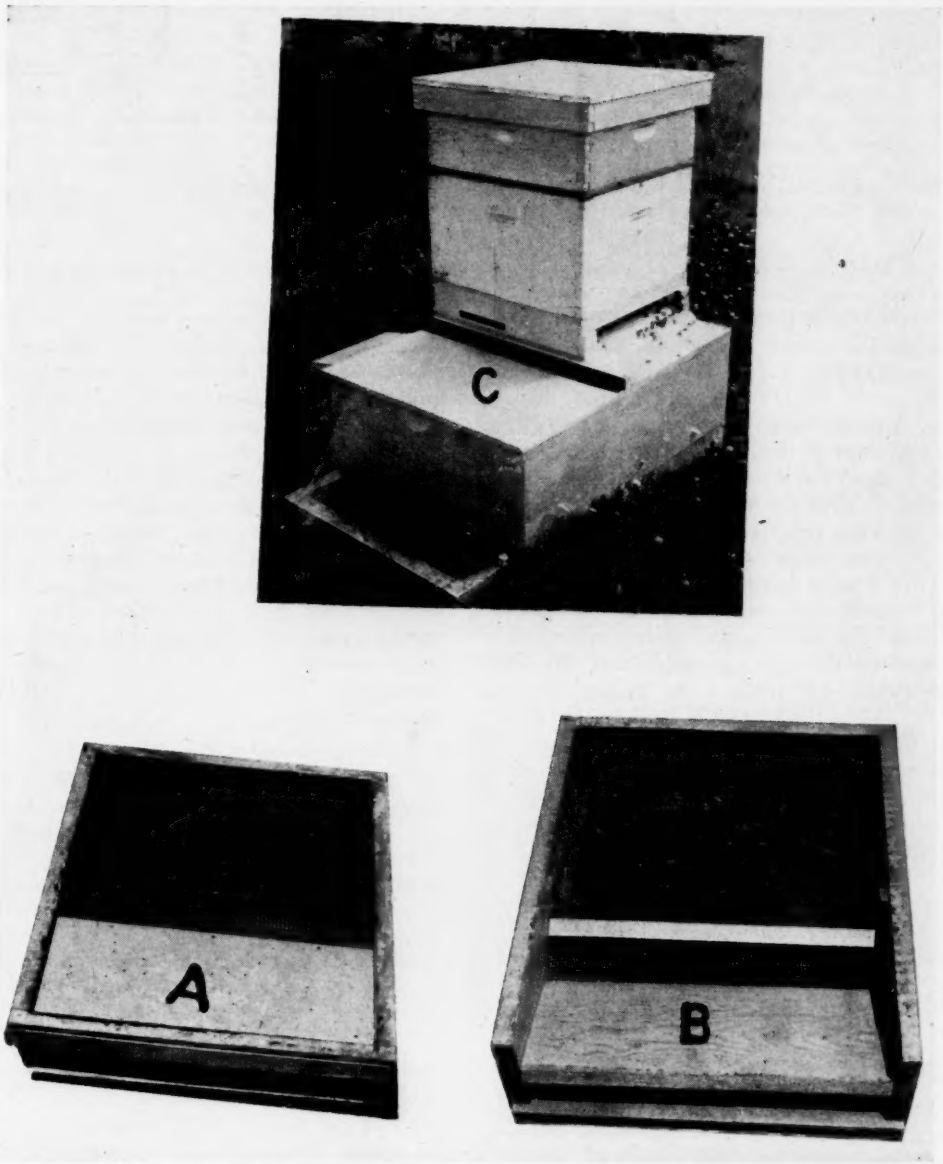
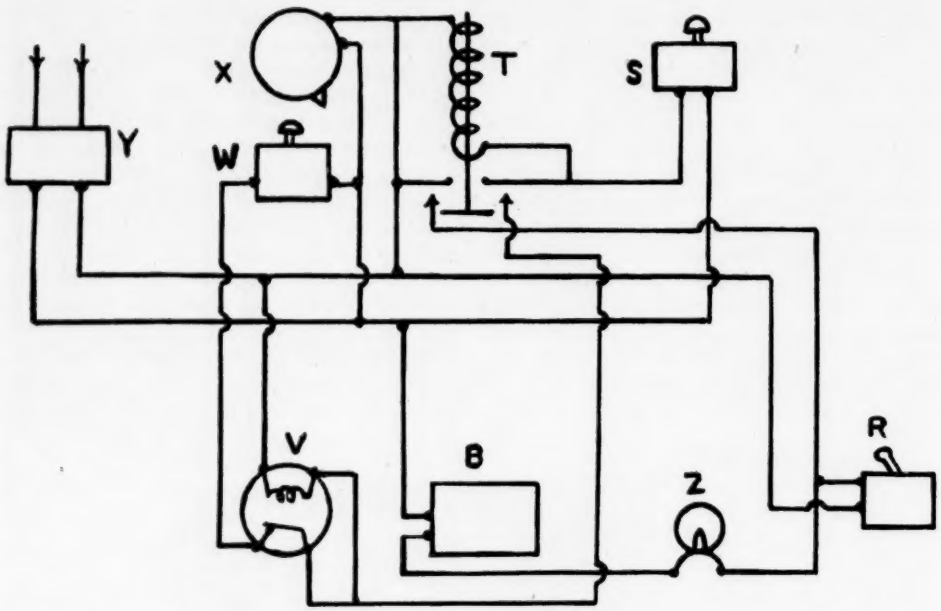


PLATE 2: A. Top view of pollen trap. B. Bottom view of pollen trap. C. Dadant hive on top of pollen trap which is on top of pollen collector.



TEXT-FIG. 1.—Electrical wiring diagram: R, toggle switch; S, microswitch; T, magnetic relay; V, relay tube, normally closed; W, microswitch; X, Haydon timer; Y, metermatic time switch; Z, electric lamp for resistance.

day activity of the bees is finished, the rotation of the turntable is required. The motor which rotates the turntable is activated by throwing the toggle switch (R) which by-passes the timing mechanism. The turntable may be stopped at the desired locations by using the toggle switch.

OPERATING THE DEVICE.—The white starting clip fixed on the periphery of the dial of the metermatic time switch (Plate 1, fig. Y) at 6 A.M. makes contact and completes the electric circuit with the Haydon timer (X) (Plate 1, fig. Z). From this time on through the day until 8 P.M., the Haydon timer controls the rotation of the turntable. After 2 hours a complete rotation is made by the Haydon timer and the pointer attached to it touches the microswitch (W), allowing the electric current to go through to activate the motor assembly unit (B) (Plate 1, fig. X), relay tube (V) (Plate 1, fig. Z), and the magnetic relay (T). Then the turntable starts rotating. In 3 seconds the filament in the relay tube (V) heats and opens, allowing the current to go through the microswitch (S) which is normally closed. This microswitch takes over control at this time. When the button on the turntable contacts the button of the microswitch (S), it opens the circuit and stops the motor. The turntable division button opens the microswitch which, in turn, opens the circuit in the magnetic relay (T), and the motor assembly unit (B) stops (Plate 1, fig. X). Every 2 hours the same cycle will be repeated until 8 P.M., when the black stopping clip fixed on the periphery of the dial of the metermatic time switch stops the mechanism operating the turntable.

The whole apparatus is enclosed in a wooden box to protect it from the weather. The device (Plate 2, fig. C) was placed beneath a pollen trap which was put under the brood chamber of an 11-frame Dadant hive. The bees in entering the hive must pass through the hardware cloth of the trap which shears off the pollen loads from their hind legs (Plate 2, figs. A and B). The pollen falls into the pans on the turntable. Each pan has a number corresponding to a particular 2-hour period of the day. In the writer's particular study, the pollen collected in each pan was weighed separately and correlated with the climatological data. Another use of this device is that partial separation of the pollen can be obtained from different sources, since anther dehiscence and pollen presentation of the different plant species occur at different periods of the day.

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Grain Storage Fungi Associated with the Granary Weevil[1]

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Certain fungi, especially those in the genus *Aspergillus*, are primarily responsible for much deterioration in stored grain, producing what is known in the trade as "germ damaged" or "sick" wheat, and other deleterious changes. The literature dealing with this has been adequately reviewed (Christensen 1957, Semeniuk 1954) and will not be gone into in detail here. Members of the *Aspergillus glaucus* "group species" are able to invade the germs of wheat at moisture contents below 15%, and members of the *A. restrictus* series, of the *A. glaucus* group species appear to be especially damaging to grain quality (Christensen 1957). In the past few years, numerous samples of grain have been obtained from commercial storage bins where germ damaged wheat had developed; some of these had light infestations of the granary weevil, *Sitophilus granarius* (L.), or evidence of past feeding of this insect. Some of these lots in which *S. granarius* was or had been present were heavily invaded by *A. restrictus*. This suggested the possibility that infestation of grain by this insect might promote invasion of the seed by *A. restrictus* or other storage fungi, and the present work was undertaken to explore this.

Materials and Methods.—*Source of insects.*—Stock cultures of *S. granarius* were raised on wheat in wide-mouth screen covered Mason jars. The wheat had a moisture content of 14 to 15% when put in the jars, and the cultures were kept at a temperature of 25° C. and a relative humidity of 75%.

Wheat.—A hard red spring wheat that had been grown in the dry land region of Montana was used. This was chosen because it contained very few storage fungi of any kind, and could be almost completely freed of these by a light treatment with sodium hypochlorite.

Moisture content was determined by the two-stage, air-oven method specified by Cereal Laboratory Methods (Amer. Assoc. of Cereal Chemists 1957), at the beginning of the tests and whenever portions of grain were removed for study. All moisture contents are given on a wet weight basis.

Mold counts were determined by the procedures developed to evaluate moldiness of grains and grain products (Christensen 1957). Essentially this measures the number of viable spores present; such quantitative data on sporulation offer one criterion of the degree to which certain fungi have invaded a given lot of grain.

Preparation of grain and of insects.—The wheat was shaken for 1 minute in a 1% solution of sodium hypochlorite to remove contaminating fungi, washed briefly in running water, dried, and the moisture content determined. If a higher moisture content was desired, sterile water was added to the grain and it was kept in a refrigerator at 5° C. and shaken occasionally for several days, to insure even distribution of water.

The adult weevils to be added to the grain were washed briefly in running water to which soap powder was added, and shaken for 1 minute in a 1% solution of sodium hypochlorite to remove or kill external spores of fungi. This treatment caused no detectable injury to the insects.

Containers.—Glass tubes 1½×4 in. in size were fitted with a cork stopper at each end; a hole ¾ inch in diameter was bored through each cork, and the inner end of the stoppers covered with a copper screen of 32 meshes per inch. Usually 40 grams of wheat were placed in each tube, and when the tubes were in a horizontal position the grain had a maximum depth of approximately ½ inch.

Maintenance of relative humidity and aeration.—The glass tubes containing grain, and grain plus insects, were kept in a desiccator in a chamber at 25° C. Air was circulated, by means of glass tubing, through two jars in series, each containing a saturated solution of sodium chloride, to give a relative humidity of 75 to 76%, and circulated through the desiccator.

Results.—*Development of fungi in wheat infested with insects compared with uninfested wheat.*—Ten, 20 and 40 weevils were added to 40-gram samples of grain at a moisture content of 12.1%. At intervals of 1 month moisture content and mold count were determined. The data obtained after storage for 3 months are summarized in table 1.

Table 1.—Moisture content and mold count of wheat infested with different numbers of granary weevils and stored 3 months at 75% relative humidity and 25° C.

Number of Weevils	Replicate	Final Per Cent Moisture Content[a]	Mold Colonies/Gram of Wheat	Percentage of *Aspergillus* Made Up by		
				restrictus	*repens*	*amstelodami*
10	1	17.6	905,000	98	2	0
	2	19.7	2,865,000	99	1	0
20	1	18.0	2,600,000	100	0	0
	2	18.7	5,050,000	98	2	0
40	1	22.7	56,700,000	100	0	0
	2	23.0	38,600,000	95	3	2
None (control)	1	14.8	2,000	0	100	0
	2	14.6	500	0	100	0

[a] Original moisture content, 12.1%.

The control samples at the end of the 3-month test yielded no cultures of *A. restrictus*, and only a low count of *A. repens* (another member of the *A. glaucus* group species). In those infested with weevils the count of *A. restrictus* ranged from slightly less than 1 million per gram of grain to almost 57 million per gram. The final moisture content of the controls ranged from 14.6 to 14.8%, which is the moisture content to be expected in hard red spring wheat in equilibrium with a relative humidity of 75% (Coleman & Fellows 1925). The increase in moisture content of the samples containing weevils was much greater than that in the controls, and was approximately proportional to the number of weevils

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originally added to the grain. The increase in *A. restrictus* in the samples where weevils were present was approximately proportional to the moisture content increase. Since the weevils and the grain had been surface disinfected before the test was begun, the *A. restrictus* apparently was introduced to the grain by the weevils. Whether the relatively tremendous increase in *A. restrictus* in those samples where the weevils were present was due mainly to increasing moisture content as a result of insect activity, could not be determined. The circulation of humidified air through the tubes evidently was too slow to carry off the excess water produced by biological activity, and where 40 weevils were present originally, the moisture content of the grain increased more than 10%.

To determine whether moisture content increase alone was chiefly responsible for the vigorous growth of *A. restrictus* in those samples where weevils were present, 400-gram lots of grain were put in flasks equipped with perforated polyethylene glycol tubing through which air of 75% relative humidity was forced at a rate of 3 liters per minute. No weevils were added to the controls, and 100 weevils were added to each of the inoculated replicates. Before being put in the bottles, the grain was surface-disinfected and conditioned to a moisture content of 14.9%, and the insects were surface-disinfected before adding them to the grain. The grain was kept at 25° C. for 6 weeks, after which it was tested for moisture content and mold count. The results are given in table 2.

The aeration was sufficient to carry off water produced as a result of activities of the insects and fungi, since the moisture content of the grain did not change. Approximately the same number of colonies of *A. repens* were obtained from the controls as from the samples infested with weevils, but more than 10 times as many colonies of *A. restrictus* were obtained from the weevil infested samples as were obtained from the controls. Presumably the surface-disinfected insects were contributing inoculum or *A. restrictus* to the grain.

To test this assumption, wheat was autoclaved to eliminate all microflora from it, and adjusted to a moisture content of 14.0%. Adult weevils were removed from the stock culture, surface-disinfected, placed in a sterile jar, and kept without food for several days, so that they had no opportunity to ingest fresh spores. Controls were kept free of insects, and eight weevils were placed on 20 grams of grain in each of the inoculated samples. All were kept at 25° C. in a desiccator through which air with a relative humidity of 85% was passed. After 6 weeks, the samples were tested for moisture content and mold count. The results are given in table 3.

Table 2.—Moisture content and mold count of wheat infested and not infested with granary weevils and kept at a moisture content of 15%, at 25° C., for 6 weeks.[a]

Treatment	Replicate	Final Moisture Content[b] (Per Cent)	Mold Colonies per Gram of Wheat	
			A. restrictus	*A. repens*
Infested with weevils	1	15.0	1,310,000	260,000
	2	15.0	1,300,000	250,000
Not infested (control)		14.9	75,000	230,000

[a] 100 adult granary weevils per 400 grams of grain.
[b] Original moisture content, 14.9%.

Table 3.—Moisture content and mold count of autoclaved wheat, infested and not infested with granary weevils, and kept for 5 weeks at 85% relative humidity and 25° C.[a]

Treatment	Replicate	Final Moisture Content[b] (Per Cent)	Mold Colonies per Gram of Wheat	
			A. restrictus	*A. repens*
Infested with weevils	1	20.6	5,360,000	1,330,000
	2	20.8	5,700,000	1,250,000
Not infested (control)		16.8	0	0

[a] Eight granary weevils per 20 grams of grain.
[b] Original moisture content, 14.9%.

The controls remained free of fungi, and the moisture content was that expected in wheat in equilibrium with a relative humidity of 85% (Coleman & Fellows 1925). The moisture content of the samples inoculated with weevils increased to slightly over 20%, and the mold count of each of the inoculated samples was slightly under 7 million per gram. Evidently even the weevils starved for some days still contained a relatively large amount of inoculum of *A. restrictus*, as well as some of *A. repens*.

Presence of storage fungi within the insect.—Adult weevils from the stock culture were washed for 2 minutes in running water to which soap powder was added, shaken for 1 minute in 1% sodium hypochlorite, rinsed in sterile 7.5% sodium chloride solution, and cultured on malt-salt agar. Approximately 200 weevils were so cultured. Within 7 to 14 days *A. restrictus* grew from nearly all of them. Usually the fungus grew out from the end of the snout or from the anal opening, occasionally from the thorax or abdomen. Typical examples are shown in figure 1.

Fifty adult weevils were dipped in sodium hypochlorite to rid them of external contaminating spores, dissected aseptically, and the gut removed and cultured on malt-salt agar. All of these yielded either *A. repens* or *A. restrictus*, or both. Frequently hyphae grew out from the entire length of the gut, but grew first or in seemingly larger numbers from the proventriculus.

Approximately 50 adult weevils were similarly dissected, and the proventriculus alone cultured on malt-salt agar, or on Czapek's agar containing 40% sucrose, a medium known to be favorable to the growth of *A. restrictus*. Some of these were cultured on hanging drops on van Tiegham cells, so that they could be observed with low and high power of the compound microscope.

Most of these yielded cultures of *A. restrictus*. Frequently a mass of hyphae grew out from the end of the proventriculus that opens into the mid gut, as shown in figure 2. A number of the proventriculi were removed from the agar medium as soon as hyphae appeared, were crushed, stained with methylene blue, and examined microscopically. These contained large numbers of germinating spores and hyphal fragments.

Adult weevils from a stock culture were surface-disinfected, and put in a small sterile test tube. Individual fecal pellets deposited by the insects were removed and

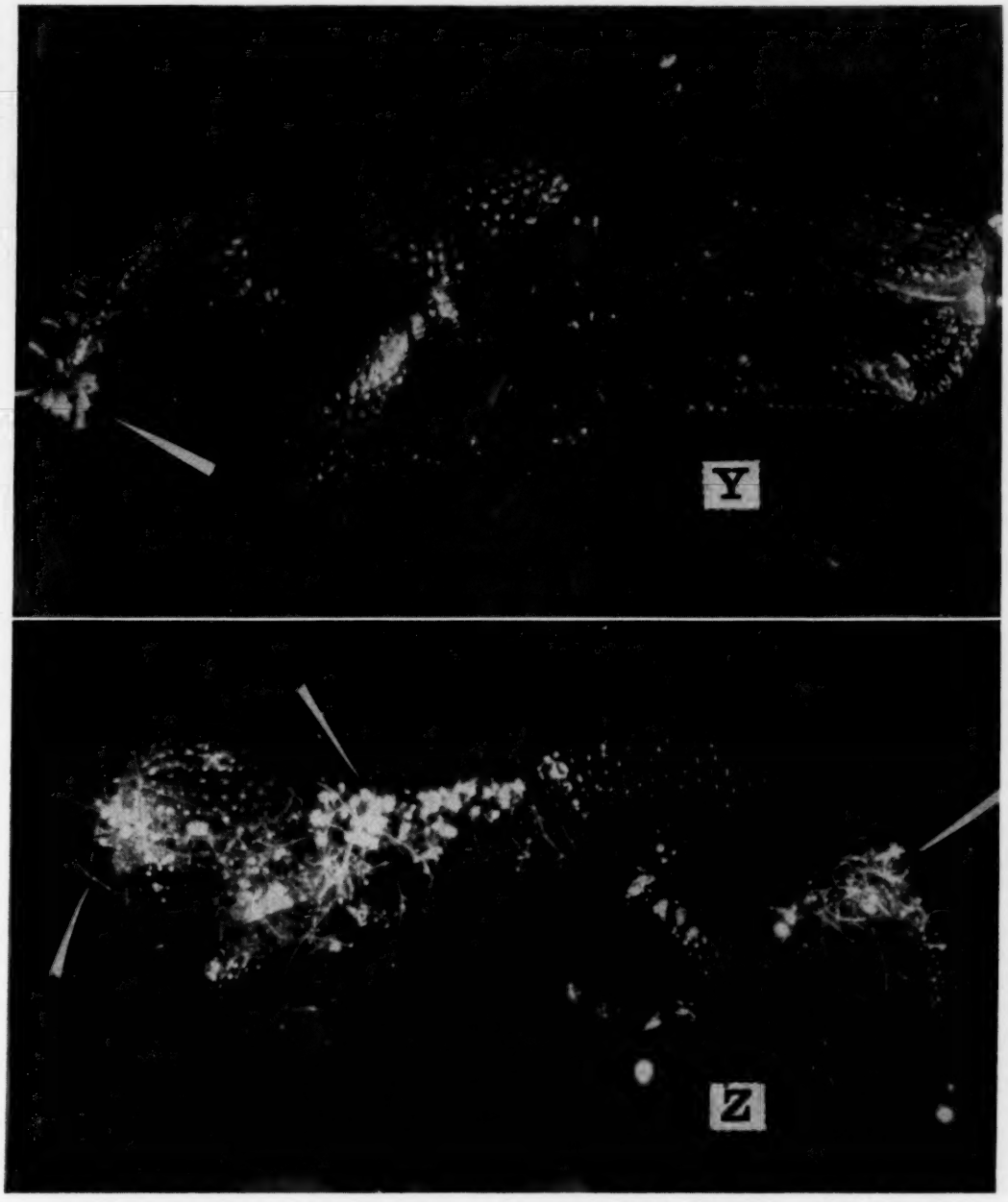

FIG. 1.—*Aspergillus restrictus* growing from insects that were surface disinfected and incubated 7 to 10 days on malt-salt agar. Y. Fungus growing from and fruiting on snout. Z. Fungus growing from and fruiting on head and abdomen. Arrows point to fungus.

crushed in hanging drops of malt-salt agar. From 2 to 14 colonies of *A. restrictus* were obtained from each pellet, evidence that spores or other inoculum survived in the feces.

One hundred adult weevils 10 to 15 days old were removed from the stock culture, surface-disinfected, placed in sterile tubes, and starved for 10 days at which time some of them died and others were obviously weakened. These were then surface-disinfected and cultured on malt-salt agar. *A. restrictus* alone grew from 58, *A. restrictus* and *A. repens* together grew from 27, and 11 yielded no fungi. This suggests that inoculum of the fungus or fungi survived in the insect as long as the insect remained alive, and that presence of inoculum of these fungi in the gut of the insect was not dependent on continual ingestion by the insect of fresh spores.

At various times, adult weevils were taken from stock cultures or from the test lots of grain and placed, without surface disinfection, on malt-salt agar or other media suitable for the growth of common storage fungi. In all cases an abundance of colonies of *A. restrictus, A. repens, A. amstelodami* and *A. ruber* (the principal members of the *A. glaucus* group found on deteriorating wheat in storage) and occasional colonies of *A. candidus, A. ochra-*

FIG. 2.—Mycelium and sporophores of *Aspergillus restrictus* growing from proventriculus of *S. granarius*. The proventriculus was dissected aseptically from a surface disinfected adult weevil, and cultured on a hanging drop of malt-salt agar.

ceus and *A. flavus* (species also known to be involved in the production of germ damaged or sick wheat) grew along the tracks left by the insect on the agar. It would be expected, of course, that if these molds were sporulating on the grain in which the insects developed, the insects would acquire a considerable load of external inoculum.

Storage molds associated with other grain infesting insects.—Larvae and adults of a number of other insects that infest grain or grain products were cultured, with and without prior surface-disinfection, on agar media favorable to the growth of common storage fungi. Most of these insects carried, both externally and internally, inoculum of some of the common storage fungi, especially those in the *A. glaucus* group, including *A. restrictus*. Additional work with the fungi on and in some of these insects is in progress.

DISCUSSION.—The source, or reservoir, of inoculum of *A. restrictus* has been something of a puzzle. Spores of the fungus have been found occasionally and sporadically in some numbers in the air in grain elevators and in grain dust collected from some terminal grain storage elevators (Tuite & Christensen 1957), but are not numerous on freshly harvested, sound wheat. It may well be that *S. granarius* and other grain infesting insects provide the primary source of inoculum of this fungus, and that even light infestations by these insects serve to provide both abundant inoculum of *A. restrictus* and provide the conditions under which the fungus can develop rapidly. Certainly the evidence accumulated in the present tests make it seem that there is a close and constant association between the granary weevil and this damaging storage fungus.

S. granarius, as well as a number of other grain infesting insects, is commonly supposed to invade grain at moisture contents well below 13 to 14%, which is the lower limit of moisture content that permits *A. restrictus* to grow. There is relatively little critical evidence to indicate that, once the insects have become established in grain whose moisture content is in the range of 12 to 13%, the moisture content remains at that level. Some of the data concerning moisture content in relation to development of insects in stored grain must be accepted with reservations. There are numerous papers in which moisture content of grain would appear to be incorrectly measured, but absence of details of method make it impossible to criticize them specifically. However, a recent paper (Daniels 1956) dealing with *Tribolium*, which does indicate the method used, states that wheat in equilibrium with a relative humidity of 75% had a moisture content of 12.2% ± 0.1%. The moisture content of the grain was determined with a Steinlite moisture meter. Well established data (1),[5] amply supported by many tests in different laboratories, including some hundreds of tests in our own work on grain storage, prove that wheat in equilibrium with a relative humidity of 75% has a moisture content of approximately 15%, not 12%. Accurate determinations of moisture contents of grain require apparatus and procedures such as those specified in Cereal Laboratory Methods (Amer. Assoc. of Cereal Chemists 1957). Even where moisture contents have been determined more accurately than those in the recent work cited above, there frequently have been no tests of moisture content of the grain after the insects have be-

[5] The data on moisture content in this paper are given on a dry weight basis, and must be converted to wet weight to agree with common usage in cereal technology.

come established in it. In our tests, reproduction and development of the insect was always accompanied by an increase in moisture content to a level where *A. restrictus* could grow fairly rapidly, and rather forceful aeration was required (with air at a relative humidity of 75%) to maintain a uniform moisture content when the insects were increasing. Tests now are under way to determine whether the weevil can develop in grain whose moisture content is kept constantly below the level where storage fungi can grow. The results obtained so far justify at least the tentative conclusion that infestations by *S. granarius* in bulk grain may at times promote and be accompanied by vigorous growth of *A. restrictus* and other injurious storage fungi.

SUMMARY.—Replicate samples of wheat infested and not infested with the granary weevil, *Sitophilus granarius* (L.), were stored for 1 to 3 months at a relative humidity of 75% and a temperature of 25° C. and tested periodically for moisture content and for fungi known to cause deterioration of stored grain. In all cases where insects increased, the moisture content of the grain increased considerably, and the mold count increased greatly, above those of the non-infested controls. The major fungus present was *Aspergillus restrictus*, known to be responsible for development of germ damaged or sick wheat. Inoculum of *A. restrictus* and of other species of the *A. glaucus* group was present in the proventriculus and intestine of the weevils, was recovered from their excreta, and persisted in starved insects until after the insects died. When surface-disinfected weevils were added to autoclaved grain, and the grain kept at a relative humidity of 85% for 5 weeks, *A. restrictus* increased greatly in the grain, but was not found in the control samples kept free of insects. It seems probable that infestations of grain by *S. granarius* may serve to inoculate the grain with storage fungi and to promote rapid growth of some of these fungi.

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Control of the Boll Weevil and Bollworm with Chlorinated Hydrocarbon and Phosphorus Insecticides in 1956[1]

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The extreme drouth in central Texas in 1956 made conditions unfavorable for the development of injurious late-season infestations of the boll weevil (*Anthonomus grandis* Boh.) and the bollworm (*Heliothis zea* (Boddie)) in the dryland cotton acreage. In the comparatively small irrigated acreage heavy bollworm and medium boll weevil infestations afforded an opportunity to conduct field-plot experiments with chlorinated hydrocarbon and phosphorus insecticides for their control. Results reported herein with one exception were obtained in experiments conducted in irrigated cotton.

PROCEDURE.—Five experiments were conducted in small plots and three in large plots. In experiments 1 and 2 the plots were 16 rows wide and of sufficient length for areas of 0.1 and 0.2 acre, respectively; those in experiments 5, 6, and 7 were 10 rows wide and 0.1 acre in size. The plots were arranged in randomized blocks except in experiment 7, where a Latin-square arrangement was used. There were four replications of treatments. In large-plot experiments 3, 4, and 8 the plots ranged from 2 to 10 acres in size. In experiment 3 all treatments except the check and toxaphene-DDT were replicated twice, but in experiments 4 and 8 none of the treatments were replicated.

Methods of applying insecticides were the same as those reported by Cowan *et al.* (1956). Applications were made at approximately 5-day intervals with the following exceptions: 6 or 7 days elapsed between the second, third, and fourth applications in experiments 2, 5, and 6; and 6 days between the third and fourth applications in experiment 1, because irrigation water was applied at that time and the fields were too wet for the use of spray equipment.

The procedure and method of recording data were the same as those reported by Ewing & Parencia (1943).

The bollworm was the insect of primary importance in all except experiment 4. In experiments 1, 2, and 3 conducted for boll weevil control, an attempt was made to remove it as a factor influencing results by treating checks with DDT and by including the same dosage of DDT with the weevil insecticides, which were not expected to give control of the bollworm (Cowan *et al.* 1956, Parencia & Cowan 1953). The only other insect of any importance was the cabbage looper (*Trichoplusia ni* (Hbn.)) and all

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[2] In cooperation with the Texas Agricultural Experiment Station.

treatments except DDT, dieldrin, Guthion, and Dipterex sprays gave satisfactory control of this pest. In experiment 1 the plots were treated with 0.6 pound of endrin on August 8 to prevent damage in the check (DDT) and spray plots. In experiment 2, to avoid use of an insecticide, such as endrin, that would influence boll weevil control, the check (DDT), Guthion, and Dipterex plots were treated with 1.5 pounds of Paris green per acre on August 10 for control of cabbage looper. Enough control was obtained to prevent much ragging of leaves in the last two treatments, but considerable damage occurred in the check. In the other experiments some ragging occurred in the checks, but it was not severe.

The insecticides used in these experiments are listed in table 1. Dieldrin-DDT, endrin, and toxaphene-DDT used as standards of control were commercial products. The other dusts and emulsifiable concentrates were experimental materials obtained from various manufacturers.

RESULTS.—In experiment 1 each insecticide reduced the boll weevil infestation significantly below that of the check. The dieldrin-DDT spray, the Guthion-DDT dust, and the 0.375-pound spray dosage of Guthion-DDT were significantly better than the 0.125-pound dosage of Guthion-DDT. The Guthion-DDT dust gave better control than any of the other treatments except the 0.375-pound spray dosage of Guthion-DDT. It gave better bollworm control than any of the other insecticides and produced a significantly higher yield. However, the other boll weevil insecticides gave a significant increase in yield over the check (DDT).

In experiment 2 there were no significant differences between treatments in boll injury by bollworms. Each insecticide except Dipterex reduced the boll weevil infestation significantly below that of the check and all except methyl parathion were better than Dipterex. Each of the other insecticides gave a significant increase in yield over Dipterex and the check (DDT).

In experiment 3 the 0.375-pound dosage of Guthion gave the best boll weevil control, and the 0.25-pound dosage was only slightly less effective. The addition of 0.5 pound of malathion to the toxaphene-DDT mixture ap-

Table 1.—Effectiveness of several organic insecticides applied as dusts and sprays against the boll weevil and the bollworm in field-plot experiments.

Insecticide (Pounds per Gallon in Sprays, Per Cent in Dusts)	Active Ingredient per Acre (Pounds)	Per Cent of—			Pounds of Seed Cotton per Acre	
		Weevil-Punctured Squares	Bollworm-Injured—			
			Squares	Bolls	Total	Gain Over Check
Experiment 1 (9 applications between July 7 and August 13)						
Sprays						
Dieldrin 1+DDT 2	0.375–0.92	25.8	11.7	6.2	2057	332
Guthion 2+DDT 2	.125– .92	35.0	11.9	7.7	1975	250
	.25 – .92	29.3	10.4	7.5	2046	321
	.375– .92	24.7	9.4	6.3	2056	331
Dusts						
Guthion 2.5+DDT 5	.40 – .81	17.0	7.6	3.5	2319	594
Untreated (DDT 2)	.92	51.2	13.0	7.8	1725	—
L.S.D. (at 5% level)	—	8.2	1.9	1.3	228	—
Experiment 2 (9 spray applications between July 7 and August 15)						
Guthion 2+DDT 2	0.25–0.97	29.0	10.3	7.4	1960	245
Dipterex[a]+DDT 2	2.0 – .97	48.6	7.8	5.3	1747	32
Methyl parathion 4+DDT 2	.5 – .97	38.8	9.2	7.6	1909	194
EPN 4+DDT 2	.5 – .97	35.9	10.2	7.5	2010	295
Endrin 1.6	.36	35.6	12.3	6.9	1930	215
Untreated (DDT 2)	.97	59.4	8.9	7.6	1715	—
L.S.D. at 5% level	—	12.0	2.4	—	106	—
Experiment 3 (9 spray applications between July 16 and August 20)						
Toxaphene 4+DDT 2	2.0–1.0	52.9	5.5	2.6	1668	538
Toxaphene 4+DDT 2-malathion 5[b]	2.0–1.0–0.5	36.7	4.0	1.7	2015	885
Guthion 2+DDT 2	0.25 –1.0	29.2	4.8	2.6	1832	702
	.375–1.0	25.7	3.8	2.2	2048	918
Untreated (DDT 2)	1.0	75.9	7.0	3.1	1130	—
Experiment 4 (4 spray applications between August 7 and 21)						
Endrin 1.6	0.3	18.6	1.7	1.6	1485	−15
Endrin 1.6+malathion 5	.3–0.5	13.8	1.2	2.4	1593	93
Toxaphene 4+DDT 2	2.0–1.0	16.8	2.5	2.3	1621	121
Toxaphene 4+DDT 2+malathion 5	2.0–1.0–0.5	11.0	2.1	2.5	1571	71
Untreated check	—	63.8	0.8	1.3	1500	—

[a] 50% soluble powder.
[b] Malathion used in last five applications.

Table 1—(continued)

Insecticide (Pounds per Gallon in Sprays, Per Cent in Dusts)	Active Ingredient per Acre (Pounds)	Per Cent of—			Pounds of Seed Cotton per Acre	
		Weevil-Punctured Squares	Bollworm-Injured—			
			Squares	Bolls	Total	Gain Over Check
Experiment 5 (8 applications between July 9 and August 13)						
Sprays						
Toxaphene 4+DDT 2	2.25–1.12	29.2	12.5	6.5	2022	910
Guthion 2+DDT 2	0.375–0.88	21.8	15.1	10.3	1862	750
Dusts						
Guthion 2.5+DDT 5	.44– .87	19.5	15.1	7.4	2203	1091
Thiodan 3	.61	20.6	17.6	10.0	2020	908
Untreated check	—	47.9	30.6	18.0	1112	—
L.S.D. at 5% level	—	9.4	4.6	2.6	125	—
Experiment 6 (8 applications between July 9 and August 10)						
Sprays						
Toxaphene 4+DDT 2	2.0–1.0	27.3	9.7	5.2	2072	974
Malathion 5+DDT 2	2.5–1.0	24.9	7.8	4.8	2101	1003
Thiodan 2	0.5	29.2	15.1	8.8	1609	511
Dust						
Thiodan 3	.53	29.4	10.8	6.3	1960	862
Untreated check	—	53.4	27.8	17.4	1098	—
L.S.D. at 5% level	—	8.9	4.8	2.1	193	—
Experiment 7 (6 applications between July 20 and August 9)						
Sprays						
Thiodan 2	0.5	14.2	11.1	7.9	1244	292
Dusts						
Endrin 2	.34	9.9	6.4	2.9	1514	562
Thiodan 3	.56	11.1	7.9	5.8	1285	333
Untreated check	—	32.9	21.1	15.2	952	—
L.S.D. at 5% level	—	9.7	7.5	7.0	90	—
Experiment 8 (4 spray applications between June 29 and July 16)						
Toxaphene 4+DDT 2	2.0–1.0	9.4	4.6	5.8	525	89
Guthion 2	0.5	7.4	5.8	8.6	426	−10
	.75	4.7	4.0	4.5	497	61
Guthion 2+DDT 2	.375–0.75	4.0	3.3	5.6	462	26
Untreated check	—	46.0	2.3	2.8	436	—

peared to improve the control. However, the infestation was higher in the last treatment, 45.5% versus 39.1% of punctured squares before malathion was added. Both the Guthion-DDT mixtures and toxaphene-DDT-malathion gave higher increases in yield over the check than toxaphene-DDT.

Experiment 4 was started in a field in which the cotton was well advanced toward the end of the fruiting period. The field received an accidental irrigation when a levee broke in the adjacent field in which experiment 3 was conducted. The addition of 0.5 pound of malathion to endrin and to the toxaphene-DDT mixture improved control slightly, but the materials alone also gave very good control. Irregular irrigation in the various plots made it impossible for yields to reflect the control obtained.

In experiment 5 each insecticide reduced the bollworm square and boll injury significantly below the check. Boll-injury records show that Guthion-DDT dust and toxaphene-DDT spray gave better bollworm control than Guthion-DDT spray and toxaphene-DDT spray was better than Thiodan. Each insecticide reduced the boll weevil infestation significantly below that of the check, and Guthion-DDT dust was better than toxaphene-DDT spray. Each insecticide gave a significant increase in yield over the check. Guthion-DDT dust, toxaphene-DDT spray, and Thiodan dust gave significantly higher yields than the Guthion-DDT spray. The difference in yield between Guthion-DDT dust and toxaphene-DDT spray was probably due to mechanical injury to plants in the latter treatment from the spray equipment used.

In experiment 6 each insecticide reduced the boll weevil and bollworm infestations significantly below the check. Boll injury records show that toxaphene-DDT spray, malathion-DDT spray, and Thiodan dust gave better bollworm control than Thiodan spray. Each insecticide gave a significant increase in yield over check, and each of the other insecticides gave significantly higher yields than Thiodan spray.

Experiment 7 was started later than experiments 5 and 6. Considerable bollworm injury had occurred, and the field received only one irrigation. There was no significant difference in boll weevil and bollworm control be-

tween insecticides, but each of them reduced the infestations significantly below the check. Each insecticide gave a significant increase in yield over check, and the endrin spray gave a significantly higher yield than Thiodan dust or spray.

Experiment 8 was conducted in unirrigated cotton. Each insecticide except Guthion at 0.5 pound per acre gave good bollworm control. Guthion at 0.75 pound and the Guthion-DDT mixture compared favorably with toxaphene-DDT. The treated plots had received toxaphene, Guthion, or Dipterex for control of the cotton fleahopper (*Psallus seriatus* (Reut.)) on June 8 and 15, and this accounts for the greater bollworm injury in them over the check. Drouth seriously affected yields, and increases were small even in the best treatments.

CONTROL BY GROWERS.—The irrigated acreage required intensive use of control measures for bollworms and to a lesser degree for boll weevils. All growers effected good control of boll weevils. Bollworm control and good yields were achieved, although some damage occurred when two spray applications by airplane had to be made during windy weather in mid-July. During that dry, hot period high winds occurred continuously for 10 days. Dieldrin-DDT and toxaphene-DDT mixtures were generally used for this control.

SUMMARY.—During 1956 experiments with chlorinated hydrocarbon and phosphorus insecticides applied as dusts and as low-volume sprays were conducted in irrigated cotton in central Texas for the control of late-season infestations of the boll weevil (*Anthonomus grandis* Boh.) and the bollworm (*Heliothis zea* (Boddie)).

In several experiments 0.25- and 0.375-pound dust and spray dosages of Guthion gave good boll weevil control. EPN and methyl parathion at 0.5 pound per acre were as effective as endrin at 0.36 pound. Dipterex at 2 pounds per acre was ineffective. Thiodan at 0.5 to 0.6 pound per acre showed promise in this control.

Guthion-DDT dust was more effective in bollworm control than approximately the same amount of toxicants applied in a spray. Thiodan dust at 0.5 to 0.6 pound per acre compared favorably with toxaphene-DDT spray, but in a spray at 0.5 pound per acre Thiodan was considerably less effective. Against an advanced infestation (larger larvae) 0.5-pound dust or spray dosages of Thiodan were less effective than endrin dust at 0.34 pound per acre.

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Control of Cabbage Loopers on Cotton with Boll Weevil and Bollworm Insecticides in Central Texas in 1956[1]

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The cabbage looper (*Trichoplusia ni* (Hbn.)) became a serious pest of cotton in central Texas in 1956 for the first time in recent years, although injurious infestations have occurred for several years in such irrigated areas of Texas as the Plains and the Pecos and the Lower Rio Grande Valleys.

Approximately 1500 irrigated acres in McLennan and Falls Counties were infested during early August. Owing to extreme drouth this was the only succulent cotton in the area. However, larvae in more than usual numbers were observed in a few fields of dryland cotton earlier in the season. The only previous record of an injurious infestation in the area since 1939 was in a report from the Waco laboratory for the week ending September 16, 1944, as follows: "One area just north of Waco in the Brazos River bottom consisting of approximately 150 acres was infested with a fairly heavy population of cabbage loopers. The cotton which was located on adjoining farms was late-planted (June) and considerable dusting with 12 to 15 pounds of calcium arsenate plus 10% Paris green was necessary to prevent stripping. A few rows in the heaviest infested spot were approximately 75% stripped and ragging occurred over most of the field. This is the largest acreage of cotton in one block that has ever been observed this heavily infested by the cabbage looper. (Observations made by Ewing.)" In the intervening years only occasional larvae were observed on cotton in the area.

When the cabbage looper infestations occurred in 1956, several field-plot experiments were in progress in irrigated cotton for the control of the bollworm (*Heliothis zea* (Boddie)) and the boll weevil (*Anthonomus grandis* Boh.). These experiments afforded an opportunity for evaluating the various insecticides against the looper under these conditions in the vicinity of Waco.

PROCEDURE.—Of the six experiments in which these studies were made the first three were set up for control of the boll weevil and the last three for the bollworm. Five of these experiments were conducted in small plots and one in large plots. In experiments 1 and 2 the plots were 16 rows wide and of sufficient length for areas of 0.1 and 0.2 acre, respectively; those in experiments 4, 5, and

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6 were 10 rows wide and 0.1 acre in size. The plots were arranged in randomized blocks except in experiment 6, in which a Latin-square arrangement was used. There were four replications of treatments. In large-plot experiment 3 the plots ranged in size from 2 to 10 acres. Treatments with the exception of the check and toxaphene-DDT were replicated twice.

Methods of applying insecticides were the same as those reported by Cowan *et al.* (1956). Applications were made at approximately 5-day intervals. Only those made in late July and August influenced cabbage looper control.

The bollworm was the insect of primary importance in all experiments. An attempt was made to remove it as a factor influencing results in experiments 1, 2, and 3 by treating checks with DDT and including the same dosage of DDT with the boll weevil insecticides that were not expected to give control of the bollworm (Parencia & Cowan 1953, Cowan *et al.* 1956). Thus information was also obtained on the control of the cabbage looper with DDT. Results obtained against the bollworm and the boll weevil in these experiments are reported in a separate paper.

Infestation records were made by counting all larvae on 10 plants selected at random within each plot on various dates. Treatments and results in the various experiments are given in table 1.

RESULTS.—In experiment 1 the Guthion-DDT dust was the only treatment that gave cabbage looper control. In spray mixtures the 0.25- and 0.375-pound dosages of Guthion gave slight control, but no appreciable control was obtained with 0.125 pound of Guthion, 0.375 pound of dieldrin, or with DDT alone. To prevent severe ragging of leaves all the plots in this experiment were treated with 0.6 pound of endrin per acre in a spray on August 8; control after 5 days averaged 74%.

In experiment 2 endrin alone and mixtures containing methyl parathion or EPN plus DDT gave significantly better control than those containing Guthion or Dipterex. The Guthion and Dipterex mixtures gave better control than DDT alone. To prevent severe ragging of leaves the plots receiving Guthion-DDT, Dipterex-DDT, and DDT alone were treated with Paris green at 1.5 pounds per acre on August 10. In the Guthion and Dipterex plots sufficient control was obtained to prevent appreciable injury, but by this time considerable ragging of leaves had occurred in the DDT plots.

Owing to foliage damage bolls opened earlier in these plots than in those having satisfactory looper control. Courtesy classing of lint samples picked from the various treatments was provided by the Agricultural Marketing Service at Dallas, Tex. All samples graded M.Lt.Sp. (Middling Light Spots). However, the staple length was 15/16 inch in the looper-damaged plots while in the others it was 1 inch. There was no difference in bollworm control between treatments, and Dipterex was no better than DDT in boll weevil control or yield. It is concluded that the difference in staple was due to looper damage. The difference in value (Government cotton loan rates) was 1.15 cents per pound. The lint yield was 600 pounds and the loss in value $6.90 per acre.

In experiment 3 the infestation was of medium intensity. As in the other experiments, no control was obtained with DDT alone. Foliage injury was greater in this large plot than in the other plots. More ragging occurred in the plots treated with 0.25 pound of Guthion than in those receiving the other treatments.

In experiment 4 each insecticide reduced the infestation significantly below the check. Toxaphene-DDT spray and Guthion-DDT and Thiodan dusts gave better control than Guthion-DDT spray.

In experiment 5 the infestation was considerably lighter than in the other experiments. Each insecticide reduced the infestation significantly below the check. Malathion-DDT and Thiodan sprays were better than toxaphene-DDT. There was no difference between the dust and spray of Thiodan.

In experiment 6 each insecticide reduced the infestation significantly below the check. There were no significant

Table 1.—Effectiveness of several insecticides against cabbage loopers in field experiments conducted for control of boll weevil and bollworm.

Insecticide (Pounds per Gallon in Sprays, Per Cent in Dusts)	Active Ingredient per Acre (Pounds)	Number of Cabbage Loopers per Plant			
Experiment 1 (9 applications between July 7 and August 13)					
Sprays		*Aug. 1*	*Aug. 8*	*Average*	
Dieldrin 1+DDT 2	0.375–0.92	6.8	11.0	8.9	
Guthion 2+DDT 2	.125– .92	7.6	8.9	8.2	
	.25 – .92	7.3	6.9	7.1	
	.375– .92	6.9	7.1	7.0	
Dusts					
Guthion 2.5+DDT 5	.40 – .81	0.8	0.7	0.8	
DDT 2	.92	8.4	10.0	9.2	
L.S.D. at 5% level	—	—	—	1.8	
Experiment 2 (9 spray applications between July 8 and August 15)					
		Aug. 9	*Aug. 13*		
Guthion 2+DDT 2	0.25 –0.97	5.2	2.0		
Dipterex[a]+DDT 2	2.0 – .97	3.6	1.3		
Methyl parathion 4+DDT 2	.5 – .97	0.6	0.9		
EPN 4+DDT 2	.5 – .97	1.2	0.7		
Endrin 1.6	.36	0.4	0.6		
DDT 2	.97	8.5	1.8		
L.S.D. at 5% level	—	2.1	—		
Experiment 3 (9 spray applications between July 16 and August 20)					
		Aug. 7	*Aug. 9*	*Average*	
Toxaphene 4+DDT 2	2.0 –1.0	1.6	1.6	1.6	
Guthion 2+DDT 2	0.25 –1.0	2.8	1.4	2.1	
	.375–1.0	2.0	0.6	1.3	
Toxaphene 4+DDT 2+ malathion 5	2.0–1.0–0.5	0.6	1.1	0.8	
DDT 2	1.0	4.0	4.3	4.2	
Experiment 4 (8 applications between July 9 and August 13)					
Sprays		*Aug. 1*	*Aug. 7*	*Aug. 13*	*Average*
Toxaphene 4+DDT 2	2.25 –1.12	1.6	2.6	1.7	2.0
Guthion 2+DDT 2	0.375–0.88	2.5	4.8	3.1	3.5
Dusts					
Guthion 2.5+DDT 5	.44 – .87	1.2	1.2	0.7	1.0
Thiodan 3	.61	1.9	1.4	1.6	1.6
Untreated check	—	6.6	9.2	5.0	6.9
L.S.D. at 5% level	—	—	—	—	0.7
Experiment 5 (8 applications between July 9 and August 10)					
Sprays		*Aug. 1*	*Aug. 9*	*Aug. 13*	*Average*
Toxaphene 4+DDT 2	2.0–1.0	1.4	1.8	1.3	1.5
Malathion 5+DDT 2	2.5–1.0	0.4	0.2	0.2	0.3
Thiodan 2	0.5	0.8	0.8	0.8	0.8
Dust					
Thiodan 3	.53	1.2	1.8	0.4	1.1
Untreated check	—	3.7	2.7	1.2	2.5
L.S.D. at 5% level	—	—	—	—	0.6
Experiment 6 (6 applications between July 20 and August 9)					
Spray		*Aug. 8*			
Thiodan 2	0.5	2.8	2.6	1.1	2.2
Dusts					
Endrin 2	.34	1.5	0.5	0.6	0.9
Thiodan 3	.56	3.6	1.7	1.8	2.4
Untreated check	—	7.6	8.9	2.7	6.4
L.S.D. at 5% level	—	—	—	—	3.1

[a] 50% soluble powder.

differences between insecticides, although a greater degree of ragging of leaves was evident in the Thiodan dust and spray plots than in those treated with endrin dust.

A disease with symptoms identical with those in cabbage looper larvae in cruciferous crops on Long Island, and attributed to a polyhedral virus as the causal agent (Semel 1956), was observed in populations in which insecticidal control was poor or omitted. Only nearly mature larvae appeared to be killed by the disease, and foliage was damaged considerably before populations were materially reduced.

CONTROL BY GROWERS.—Several growers were advised on cabbage looper control, and observations were made on irrigated farms in the area. Dieldrin-DDT mixtures used in a regular schedule of spray applications by airplane for the control of bollworms and boll weevils failed to control cabbage loopers. A change to the toxaphene-DDT mixture or endrin was made to obtain control. Where 2 pounds of toxaphene plus 1 pound of DDT per acre was used, satisfactory control was maintained. Control along field edges, turn rows, or near trees was considerably poorer than that obtained in the open field.

SUMMARY.—The cabbage looper (*Trichoplusia ni* (Hbn.)) was a serious pest of irrigated cotton in central Texas in 1956. Previously an injurious infestation was observed on a small acreage in September of 1944. The effectiveness of various insecticides used in field experiments conducted for the control of the boll weevil and the bollworm was evaluated for cabbage looper control.

Good control of loopers was effected with toxaphene-DDT, methyl parathion-DDT, and EPN-DDT spray mixtures and with endrin dust or spray. Thiodan dust or spray also showed promise. Guthion-DDT dust was more effective than approximately the same amount of toxicants applied as a spray. DDT was ineffective and spray mixtures of Guthion, dieldrin, or Dipterex with DDT gave poor control. Severe foliage damage by the pest resulted in a reduction of staple length.

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Transmission of Peach Yellow Leaf Roll Virus by *Fieberiella florii* (Stål) and a New Vector, *Osbornellus borealis* DeL. & M.[1]

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Peach yellow leaf roll virus is a highly virulent strain in a virus complex known as cherry buckskin and western X-disease.

The following leafhopper species have been reported previously as vectors of the virus strains in this complex: *Colladonus geminatus* (Van D.) (Wolfe *et al.* 1950, Kaloostian 1951, Jensen *et al.* 1952, Jensen & Thomas 1954, Nielson & Jones 1954), *C. montanus* (Van D.) (Wolfe 1955a), *Scaphytopius acutus* (Say), *Fieberiella florii* (Stål), and *Keonolla confluens* (Uhl.) (Anthon & Wolfe 1951). Thus far there is no evidence that a vector species may transmit one strain of the virus and fail to transmit other strains.

In the eastern half of the United States a similar virus exists under the name X-disease or eastern X-disease of peach. A single vector, the saddled leafhopper, *Colladonus clitellarius* (Say), has been reported for this virus (Gilmer 1954).

The host ranges of the viruses in the western X-disease complex were assumed to be restricted to the genus *Prunus* until four of the strains occurring in California were transmitted experimentally to celery (*Apium graveolens*) by means of *C. geminatus* (Jensen 1955, 1956). This work also indicated that celery is a better virus source plant for the leafhopper vectors than peach.

The present paper reports experimental transmission and retention of peach yellow leaf roll virus by *F. florii* and field evidence that this species may be the most important economic vector of the virus in one of the main peach producing areas of California during certain years. *Osbornellus borealis* DeL. & M. is here reported for the first time as a vector of virus in the western X-disease complex.

PROCEDURE.—All vector tests were conducted in the greenhouse at Berkeley, Calif. The test insects of both *F. florii* and *O. borealis* were collected as nymphs in the field and brought to the greenhouse where they fed on infected peach trees for 31 or 45 days. The yellow leaf roll plants used as the virus source were Lovell peach seedlings grown in the greenhouse and infected by means of *C. geminatus*.

The test plants consisted of Lovell peach seedlings and celery plants, *Apium graveolens*, Var. Golden Self Blanching grown in the greenhouse. When fed upon by the test insects the plants were growing in 4 to 6 inch pots and varied in height from 6 to 15 inches. A large number of peach and celery control plants remained healthy throughout the experiments.

FIEBERIELLA FLORII.—*Biology and field observations.*—This large leafhopper was introduced from Europe and, according to Oman (1949), is the only member of the genus recorded from the Nearctic region. It is now widely distributed across the United States. This species was shown by Severin (1947) to be a vector of western aster

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yellow virus and by Anthon & Wolfe (1951) to transmit western X-disease virus in Washington.

F. florii appears to be very sporadic in its occurrence. Nymphs have been recorded from *Myrtus communis* and *Cotoneaster pannosa* (DeLong & Severin 1947) in California and from sweet cherry in the orchard areas of central Washington (Wolfe 1955c). Wolfe (1955b) also reported that in the greenhouse the developmental period from first instar nymph to adult was as long as 131 days for certain individuals. Because of this long nymphal development, *F. florii* was shown to transmit virus while still in the nymphal stage despite an assumed virus incubation period of 30 or more days in the body of the leafhopper vector. Beirne (1956) reported that eggs of *F. florii* had been found in plum root stock imported from France. He listed the following host plants: Plum, currant, sour cherry, privet and spiraea. In California it has been encountered only occasionally and when searched for it has been found consistently in only a few places. Because of its apparent scarcity and because groups of 1 to 50 were reported by Anthon & Wolfe (1951) to transmit western X-disease virus from cherry and peach to only 3 of 17 peach trees tested, *F. florii* has been considered of negligible economic importance as a vector of this virus. However, in June, 1955, a peach orchard was found south of Yuba City, in one of the main peach districts of California, having an unusually large increase in the number of diseased trees (Schlocker 1956) and harboring *F. florii* in relatively large numbers. In 1954, seven new cases of peach yellow leaf roll disease had been found in this orchard. In 1955 the new cases increased by 45 trees. This was the only orchard in Sutter County with such a large increase in virus infections, the disease having declined generally during the past few years. Most of the diseased trees were grouped together, rather than being scattered throughout the orchard. On June 16, 1955, nymphs in all stages of development and an occasional teneral adult were found on many of the peach trees in the orchard. However, not a single specimen of *F. florii* was found in adjacent orchards or in other localities in the region.

The orchard in question was sprayed with DDT by means of an airplane on June 25, 1955. On July 20, 1955, one specimen of *F. florii* was collected by sweeping the foliage of trees in this orchard.

In 1956, 129 new cases of peach yellow leaf roll were found in this orchard plus 21 diseased trees in adjacent plantings (Schlocker 1957). On May 24, 1956, small nymphs of *F. florii* were found on sweet clover plants growing against the trunks of the peach trees in this orchard and some nymphs were moving into the trees as the clover dried up. All other cover plants had been disked under. On May 30, the sweet clover plants and trunks of the trees were sprayed with DDT and on June 2 the entire orchard was sprayed with parathion by means of ground equipment. On June 6 no leafhoppers were found. In 1956, as in 1955, *F. florii* was not found in any other locality in Yuba or Sutter counties. Moreover, except for the 150 trees at this one focus of disease, only 60 new tree cases of yellow leaf roll disease were found in the entire area of Sutter, Butte and Yuba Counties (Schlocker, 1957). These circumstances indicate that in this orchard *F. florii* was the insect vector primarily if not solely responsible for the rapid increase in disease.[3] This conclusion is strengthened by the fact that *C. geminatus*, formerly considered the major economic vector of the area, has occurred in such low numbers during the last few years that not a single specimen was taken, even in alfalfa fields, during some of the monthly surveys.

In Green Valley, Solano County, California, another strain of the virus has been spreading rapidly in cherry orchards. In this area, during 1956 at least, *F. florii* appears to have played little if any part in the spread of the virus. No specimens of *F. florii* were collected by sweeping or on 12 yellow sticky boards maintained from May 10 to July 1, 1956, in the cherry orchard having the most rapid spread of the virus. However, a total of 1 to 5 *C. geminatus* was caught by the sticky boards during each 7 to 14-day exposure throughout the sampling season. These numbers though small, indicate an orchard population of dangerous magnitude if even a small percentage of the leafhoppers are viruliferous.

F. florii nymphs, together with nymphs of *O. borealis*, were collected on myrtle, *Myrtus communis*, on the grounds of Napa Junior College, Napa Calif., and on a hedge of California privet, *Ligustrum ovalifolium*, at Rutherford, Calif., on May 29, 1956. These leafhoppers were not found on the same host plants in other localities in Napa Valley or in other areas.

When caged on young peach trees in the greenhouse, adults of *F. florii* fed almost exclusively on the peach stems rather than on the leaves. They always fed with the head directed down the stem. They held onto the stem with the prothoracic and mesothoracic legs and extended the metathoracic legs out along the abdomen. The long axis of the body formed an angle of 45° to 70° with the stem on which the leafhopper rested.

Vector tests, 1955 Experiment.—Nymphs of *F. florii* in various instars were collected from both healthy and diseased peach trees near Yuba City on June 16, 1955, and were caged on infected peach trees in the greenhouse. After 31 days, 40 adults and 19 last instar nymphs were alive. The adults were caged in groups of 5 on each of 8 test peach trees (test groups 1 to 8 in table 1) for successive transfers to peach and celery. The 19 nymphs were left on the inoculum plant until all had become adults, giving them a total acquisition feeding period of 45 days. They were then caged in 3 groups of 5 and 1 group of 4 adults on test peach trees (groups 9 to 12 of table 1) on the same date that groups 1 to 8 were transferred to the second series of test peach trees. The survivors on the test trees were subsequently combined into two groups for serial transfers on celery (table 1). Feeding time of groups 1 to 8 on the peach trees and on the first two celery plants was 14, 10, 18 and 21 days, respectively. At the end of this time seven leafhoppers survived for transfer to the final celery plant upon which all leafhoppers died after effecting virus transmission.

All 5 test celery plants were infected but only 7 of 20 peach trees developed disease symptoms. The mean number of leafhoppers per test celery and peach was 6 and 4.1, respectively.

1956 Experiment.—Young nymphs of *F. florii*, in asso-

[3] In June and July, 1957, no *F. florii* were found in the above orchard and only three new cases of disease occurred. These results suggest that the 1956 control measures had killed nearly all of the vectors before they were able to transmit virus and before they could reproduce. This adds further evidence that the previous rapid spread of the virus in this orchard was due to *F. florii*.

Table 1.—Serial transmission of peach yellow leaf roll virus from peach to celery and peach by means of *Fieberiella florii* following acquisition feeding periods of 31 days (groups 1 to 8) or 45 days (groups 9 to 12), in 1955.

Group Number	Peach		Peach		Celery		Celery		Celery	
	Number of Insects	Results	Number of Insects	Results	Number of Insects	Results	Number of Insects	Results	Number of Insects	Results
1	5[a]	−	2	+						
2	5	−	4	−						
3	5	−	3	−						
4	5	+	2	+	7	+	5	+		
5	5	−	3	−						
6	5	−	2	−						
7	5	+	3	−						
8	5	+	3	−					7	+
9			5	−						
10			5	+						
11			5	−	6	+	5	+		
12			4	+						

[a] Feeding time of groups 1 to 8 on the peach trees and on the first two celery plants was 14, 10, 18, and 21 days respectively. Leafhoppers died on the terminal celery plant.

ciation with nymphs of *O. borealis*, were collected as noted above on myrtle and on California privet. Nymphs of the two leafhopper species were caged together on an infected peach tree where they fed for 45 days. Each of two groups of 6 adults and 1 group of 5 adults of *F. florii* were then caged successively on 9 celery test plants and on either 3 or 4 peach trees until all insects had died (table 2). The feeding time on each celery plant and on the first peach tree was either 3 or 4 days. Time on each of the other peach trees was 7 days.

The 3 groups of leafhoppers transmitted virus to 35 of 36 test celery plants but to only 1 of 10 peach trees. The mean number of leafhoppers per test was 5.2 compared to 2.4 per peach tree.

VIRUS RETENTION.—In the 1955 tests, virus was transmitted to the terminal celery test plant by the leafhoppers at least 63 days after leaving the virus source plant. In the 1956 experiment the last plant to be infected was fed upon at least 36 days after the leafhoppers were removed from the inoculum plant. It is possible that some of the leafhoppers were viruliferous for an even longer period but failed to demonstrate infectivity on the peach trees which apparently are less susceptible to infection by means of these leafhoppers than are celery plants.

Table 2.—Serial transmission of peach yellow leaf roll virus to celery and peach by *Fieberiella florii* adults after 45 days on infected peach.[a]

Test Plant	Test Groups of Insects and Results					
	Good A		Group B		Group C	
	Number of Insects	Results	Number of Insects	Results	Number of Insects	Results
Celery	6	+	6	+	5	+
Celery	6	+	6	+	5	+
Celery	6	+	6	+	5	+
Celery	6	+	6	+	5	+
Celery	6	+	5	+	5	+
Celery	5	+	5	+	5	+
Celery	4	+	5	+	5	+
Celery	4	+	5	+	5	+
Celery	4	+	4	−	5	+
Peach	4	—	4	—	5	+
Peach	1	—	3	—	2	—
Peach	1	—	2	—	1	—
Peach	1	—	0	0	0	0

[a] Feeding time on each celery plant and the first test peaches was 3 to 4 days. Time on other test peach trees was 7 days.

OSBORNELLUS BOREALIS.—The genus *Osbornellus* is Neotropical and Nearctic in distribution with 26 species listed by Oman (1949) for the Nearctic region. Most of these occur in the southwestern part of the United States, but *O. borealis* is a species primarily of the Northwest. Wolfe (1955c) recorded the species on *Ceanothus velutinus* and sweet cherry in the State of Washington.

In California, the writer collected *O. borealis* as young nymphs together with nymphs of *F. florii* on myrtle, *Myrtus communis*, at Napa, and on a hedge of California privet, *Ligustrum ovalifolium*, at Rutherford, May 29, 1956. The ratio of *F. florii* to *O. borealis* was 2 to 1 at Napa and 1 to 2 at Rutherford.

Nymphs of the two species are similar in general appearance, but can be distinguished by the following characteristics: In the same instars, nymphs of *O. borealis* are conspicuously smaller than *F. florii*, lack the pair of slender setae which occur on the lateral margins of abdominal segments 3 through 8, and also lack the scattered black dots on the dorsal surface of the abdomen which are present on *F. florii*. *O. borealis* nymphs are further distinguishable by the presence of a black band on the margin of the head, a black patch laterally on the thorax back of the eyes, black areas near the apex of the femur and the base of the tibia of the meta-thoracic legs and distally on the femur of the meso-thoracic legs. These dark markings are lacking on *F. florii*.

The adults of *O. borealis* are approximately 5 mm. long compared with 7 mm. for *F. florii*.

VECTOR TESTS.—*O. borealis* has not previously been reported as a vector of any plant virus. However, it occurs in the tribe Deltocephalini which includes most of the known vectors of aster yellows virus, but excludes

Macrosteles fascifrons (Stål), the most important carrier of this virus.

Nymphs of *O. borealis* and *F. florii* were caged on an infected peach tree in approximately equal numbers. At the end of 45 days on inoculum, only eight adults of *O. borealis* were alive compared with 17 adults of *F. florii*. The *O. borealis* adults were caged in three groups for successive feedings on healthy celery and peach plants (table 3).

Seven of nine celery plants and one of four peach trees, fed on by *O. borealis* adults, developed disease symptoms. Two of the celery plants infected were subsequently used as virus source plants from which non-infective *Colladonus montanus* recovered virus and transmitted it to healthy peach and celery plants.

DISCUSSION.—Field evidence acquired during 1955 and 1956, together with experimental transmission of peach yellow leaf roll virus by means of *F. florii*, suggests that this leafhopper is an important vector in sporadic outbreaks of virus in a large canning peach area of California. During these 2 years, *F. florii* was found only in one large block of peach trees but it occurred there in relatively high numbers. Moreover, only in this orchard was there a marked increase in the number of diseased trees. Furthermore, many of the new cases were grouped in close proximity to positions in which diseased trees had been found the previous year. This cluster type of disease incidence would be expected in a situation in which the vectors passed much of their nymphal development on the diseased tree and were ready to transmit virus as soon as the adults moved to nearby trees. Careful inspection records reveal that such foci or "hot spots" have occurred more than once in the area during past years.

These localized foci of disease and vector permit application of an intensive vector control program in specific orchards with a better prospect of suppressing the disease then is possible where the infected trees are more widely scattered and result from inter-orchard movement of vectors probably originating outside the orchard.

Although *Colladonus geminatus*, heretofore considered the most important vector in the area, could theoretically account for any type of spread pattern, it has not been collected on peach trees except as isolated individuals. These appears to have been produced in alfalfa fields where the species can be found most easily. However, even in alfalfa *C. geminatus* has been present in only low numbers during recent years. Movement of *C. geminatus* from alfalfa to peach orchards would be by scattered individuals which might be expected to leave a more random pattern of disease spread over a wider region rather than a cluster type in one orchard with no disease in the surrounding area.

It is suggested that *F. florii* may have been responsible for some or all of the "hot spots" of disease in Yuba and Sutter Counties, with *C. geminatus* contributing to the more widely scattered pattern of disease spread.

Earlier tests with *C. geminatus* (Jensen 1956) demonstrated that infected celery is an excellent source of peach yellow leaf roll virus. Experiments with *F. florii* (tables 1, 2) and *O. borealis* (table 3) in the present study suggest that celery is also more susceptible to infection by means of these leafhoppers than peach.

Table 3.—Transmission of peach yellow leaf roll virus to celery and peach by *Osbornellus borealis* after 45 days on infected peach.[a]

	Test Groups of Insects and Results					
	Group 1		Group 2		Group 3[b]	
Test Plant	Number of Insects	Results	Number of Insects	Results	Number of Insects	Results
Celery	3	—	3	+	3	+
Celery	2	—	3	+	3	+
Celery	1	+	3	+	3	+
Peach	1	+	1	—	3	—
Peach	1	—				
Totals		2/5		3/4		3/4

[a] Leafhoppers fed for 7 days on each of the test celery plants and 5 days or less on the test peach trese.

[b] The 3 insects in group 3 consisted of two adults of *O. borealis* plus 1 nymph which proved to be a female of *Colladonus* sp.

SUMMARY.—Field studies during 1955 and 1956 indicate that *Fieberiella florii* (Stål) may be a highly important leafhopper vector of peach yellow leaf roll virus in a major canning peach district of California. Although encountered only infrequently in the field, *F. florii* occurred in relatively high numbers in one large peach orchard 2 years in succession but was absent from all other orchards sampled in the area. In this locality the number of diseased trees increased from seven in 1954 to 202 in 1956 while disease spread in the balance of Yuba and Sutter Counties was low and occurred as widely scattered individual trees.

In greenhouse experiments, *F. florii* transmitted peach yellow leaf roll virus from peach to 40 of 41 celery plants and to 8 of 30 peach trees.

Osbornellus borealis DeL. & M., previously not recorded as a vector of any plant virus, experimentally transmitted this virus from peach to 7 of 9 celery plants and to 1 of 4 peach trees. This species was collected from *Myrtus communis* and *Ligustrum ovalifolium* in its early nymphal stages together with nymphs of *F. florii*.

Experiments with *F. florii* and *O. borealis* suggest that celery is much more susceptible than peach to infection by means of these vectors.

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Insecticides for Pink Bollworm and Boll Weevil Control in the Lower Rio Grande Valley in 1955 and 1956[1]

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Field-plot insecticide tests were continued in the Lower Rio Grande Valley in 1955 and 1956 in further efforts to develop more satisfactory measures for control of the pink bollworm (*Pectinophora gossypiella* (Saund.)), the boll weevil (*Anthonomus grandis* Boh.), and other associated cotton insects and spider mites.

Three series of randomized field-plot tests were conducted in 1955 and four in 1956. Both dusts and low-volume sprays were used. The sprays were emulsions applied with a 6-row tricycle-type self-propelled sprayer equipped with either two or three cone-type nozzles per row so as to apply, respectively around 5.0 and 7.5 gallons of spray per acre at 60 pounds pressure. Rotary hand guns were used to apply the dusts. The treatments were applied at 4- or 5-day intervals, insofar as possible, except for series 1 and 3 in 1955 where a weekly period was used for the most part. Applications washed off by rain within 24 hours were repeated as soon thereafter as conditions would permit.

A summary of the poison applications, infestation records, and yields for each series for the 2 years is given in table 1.

TESTS IN 1955.—*Series 1.*—This series consisted of four tests with two ½-acre spray replicates for each test, or a total of eight replicates. Mixtures of Guthion plus DDT and endrin plus DDT were used.

Both treatments gave noticeable reductions of the boll weevil infestations. The pink bollworm infestation was too light for any definite conclusions to be drawn. Spider mites increased enough to require control measures in the endrin-DDT plots and were of some concern, along with aphids, in part of the Guthion-DDT plots.

Both treatments gave fair increases in yield over the untreated plots, mostly owing to control of the boll weevil.

Series 2.—This was a dust series and consisted of two tests with two plots each, or a total of four replicates. The two treatments used were endrin plus DDT and sulfur, and Guthion alone. The plots ranged from 0.33 to 0.87 acre in size. No yield records were obtained.

Both treatments gave appreciable reductions of the pink bollworm populations, the best results being obtained from the Guthion.

Boll weevil infestation counts were not sufficient in this series to be included. Populations of other insects were also too low for records to be secured.

Series 3.—This was also a dust series and consisted of only one test, with one 0.15-acre plot for the untreated and one 0.31-acre plot for Guthion alone.

High infestations of both pink bollworms and boll weevils developed in this experiment, and very good control of both insects was obtained from 5% Guthion applied at approximately weekly intervals.

The increase in yield over the untreated plot was outstanding, as might be expected from the good control obtained of both the pink bollworm and boll weevil under high infestation conditions. The gain was about equally due to the control of the two insects. No other insects occurred in sufficient numbers to cause concern.

TESTS IN 1956.—*Series 1.*—This group consisted of two spray tests with two 0.6-acre plots each, or a total of four replicates, with Guthion plus DDT and Phosdrin plus DDT.

Guthion gave a noticeable reduction of the boll weevil populations, whereas Phosdrin failed to give any appreciable control.

A light infestation of pink bollworms occurred, and each treatment gave effective control of this insect.

In one of the tests bollworms caused noticeable injury

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Table 1.—Poison applications, seasonal infestation averages, and yields for pink bollworm and boll weevil control tests in the Lower Rio Grande Valley, 1955 and 1956.

Treatment	Pounds per Acre	Pink Bollworm: Per Cent of Bolls Infested	Pink Bollworm: Number of Larvae per Boll	Boll Weevil Infestation (Per Cent)	Pounds of Seed Cotton per Acre: Total	Pounds of Seed Cotton per Acre: Gain
Tests in 1955						
Series 1—sprays (8 plots,[a] 7 applications)						
Untreated		8.1	0.15	42.0	1548	—
Guthion+DDT	0.33[b]+0.50	3.2	.07	25.4	1824	276
Endrin+DDT	0.33[b]+0.50	6.1	.10	17.4	1814	266
Series 2—dusts (4 plots, 6 applications)[c]						
Untreated	—	60.4	2.41	—	—	—
Endrin 2.5%+DDT 10%+sulfur	23.5	34.7	.86	—	—	—
Guthion 4%+inert	19.7	24.4	.46	—	—	—
Series 3—dust (1 plot, 10 applications)[c]						
Untreated	—	53.2	1.40	90.6	668	—
Guthion 5%+inert	24.6	1.3	.02	14.6	2702	2034
Tests in 1956						
Series 1—sprays (4 plots, 7 applications)[d]						
Untreated	—	12.5	.23	77.5	1610	
Guthion+DDT	0.55[e]+1.0	.5	.01	32.1	2640	1030
Phosdrin+DDT	0.55[e]+1.0	1.8	.02	63.3	2120	510
Series 2—sprays (3 plots, 6 applications)[f]						
Untreated	—	0		82.0	1307	
Guthion+DDT	0.49[e]+0.50	0		37.0	1950	643
Endrin+DDT	0.49[e]+0.50	0		30.8	2283	976
Series 3—dusts (4 plots, 11 applications)[d]						
Untreated	—	41.3	1.34	41.4	1813	
3% Guthion+10% DDT and inert	26.4	6.1	.13	19.0	3097	1284
3% Guthion and inert	27.9	4.5	.09	14.8	3083	1270
Series 4—dusts (3 plots, 11 applications)[d]						
Untreated	—	0		95.3	933	
2% endrin and sulfur	30.0	0		32.1	2793	1860
Calcium arsenate	23.2	0		75.0	2243	1310

[a] Yields for 6 plots only.
[b] Range 0.25 to 0.375 pounds.
[c] One application washed off in series 3, and in two plots of series 2.
[d] One application washed off in series 4 and in two plots of series 1; also 1½ applications in series 3.
[e] Range 0.25 to 0.75 pound in series 1, and 0.25 to 0.60 pound in series 2.
[f] Does not include two dust applications of Guthion and endrin applied when plots were too wet for operation of sprayer.

to the squares at the time of the first poison application, but after that they were not of much importance. No other insects were present in sufficient numbers to cause concern.

The increases in yield over the untreated ranged from fair (510 pounds) for the Phosdrin-DDT to good (1030 pounds) for the Guthion-DDT.

Series 2.—This series was composed of only one spray test with three 0.5-acre replicates of Guthion plus DDT and endrin plus DDT. The last two applications were made with 3% dust for the Guthion and 2% dust for the endrin since the plots were too wet for operation of the sprayer.

Both the endrin and the Guthion gave effective control of the boll weevil, which was the main insect of concern in this series.

The only other insect of importance was the bollworm, of which there was a light infestation at the time the test was started.

The best increase in yield was secured from the endrin plus DDT, with a total of 976 pounds of seed cotton per acre over the untreated, as compared with 643 pounds for the Guthion-DDT.

Series 3.—This was a dust series and consisted of two tests with three 0.25-acre replicates of Guthion plus DDT and Guthion alone. Both the pink bollworm and the boll weevil were of importance in this series, especially in one of the tests.

The 3% Guthion was very effective against the pink bollworm, both with and without DDT. It was also effective against the boll weevil. No other insects occurred in sufficient numbers to be of significance.

The increases in yield were about equally good for both treatments—1,284 pounds of seed cotton per acre over the untreated for the Guthion plus DDT, and 1,270 pounds for the Guthion without DDT.

Series 4.—This was another dust series and consisted of one test with three 0.25-acre replicates of endrin plus sulfur, and calcium arsenate alone. An unusually heavy boll weevil infestation occurred during the entire poison-

ing program. Satisfactory control of the populations was obtained from the endrin plus sulfur, whereas the results from the calcium arsenate were not as good as expected.

All the plots were treated once with demeton for spider mites and aphids shortly after the first poison application, and this kept the infestations under control during the rest of the season, except for the recurrence of the aphids in the calcium arsenate plots near the end of the period. No other insects were numerous enough to be of importance.

The increases in yield over the untreated plots ranged from 1,860 pounds of seed cotton per acre for the endrin plus sulfur to 1,310 pounds for the calcium arsenate.

The Occurrence of Resistance to TDE (DDD) in the Red-Banded Leaf Roller[1]

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Since 1946, the red-banded leaf roller, *Argyrotaenia velutinana* (Wlk.), has become an important pest of apple in eastern United States. It is believed the substitution of DDT for lead aresenate for codling moth control directly and indirectly brought about this situation (Glass & Chapman 1952). The problem has become so general that most New York apple growers now regularly provide for the control of the species in their spraying operations. TDE has been the most widely used for this purpose since 1949. In certain problem orchards this material has been employed consistently, whereas in others it has been used only intermittently.

Originally, TDE gave spectacular control of the red-banded leaf roller. While it has been necessary to obtain thorough coverage when employing this insecticide, satisfactory control was usually obtained under normal orchard spray practices. Results with TDE were much superior to those obtained with other materials available in 1949.

In 1954, one grower near Sodus, N.Y. (Kelley Orchard) failed to obtain satisfactory control of the second brood with the usual single TDE spray treatment. At that time failure was attributed to faulty coverage in thick, dense trees. However, in 1955 this grower obtained only fair control in some parts of his orchard with 6 to 9 TDE sprays, some of which were very carefully applied. In 1956 he continued to have difficulty and supplemented the action of TDE through the addition of parathion and lead arsenate. A few growers other than Mr. Kelley also reported unsatisfactory control of the leaf roller with TDE in 1955, and by 1956 the number of trouble orchards increased. A few of these plantings suffered heavy fruit injury in spite of the multiple use of TDE sprays, some of which were applied at two or three times the recommended dosages. The percentage of orchards reporting difficulty in controlling the red-banded leaf roller with TDE is still quite small. It perhaps represents 5 to 10% of the apple orchards in western New York.

In the Chalupa orchard the grower used no DDT until 1954 and no TDE until the spring of 1956. The second brood of the leaf roller caused heavy losses here in 1955 yet a single TDE spray applied in the usual commercial manner gave virtually 100% control of first brood in 1956. This experience indicates that recent failures with TDE in certain orchards were not the result of unusual seasonal conditions.

Red-banded leaf roller activity has always been erratic and consequently it has been difficult to draw conclusions based on commercial control experiences. However, a review of the over-all situation in 1955 indicated the possibility of TDE resistance in the species. To determine whether this was the case, field experiments were conducted in the Kelley orchard in 1955 and 1956, and also in other plantings the second season. In addition, laboratory comparisons were made of the response of three strains of leaf roller to TDE and parathion.

FIELD TESTS.—Field tests have been conducted in New York against one or both broods of the red-banded leaf roller during most seasons since 1948. The method of application, timing in relation to insect development, and methods of assaying results have been constant. Details on procedures for these tests are given by Glass & Chapman (1952). Both TDE and parathion were included in these tests so there is a basis for making comparisons of the response of leaf roller to these materials over the period of 1948 through 1956. In some instances the same orchards were used for tests in different years.

The results of first brood tests are given in table 1 and for second brood in table 2. The data show that TDE performed very well in all tests up through 1954. Even ½ pound of wettable powder, 50% material, gave good control in 1949. In 1955 control was not quite as good as was expected based on earlier experience. In 1956, however, control was definitely down at the 1-pound and the ½-pound concentrations, was not satisfactory at 2 pounds in one orchard and only fair in the other. Results with parathion were quite consistent throughout except in the Sodus Fruit Farm in 1956 but even here it was superior to TDE.

Results obtained with TDE against the second brood show the same trend as for the first brood. From 1948 to 1952 control approached 100% whether the material was applied once or twice and at either 1 or 2 pounds of 50% formulation per 100 gallons. In 1956, however, control was not satisfactory in any of the combinations employed. Lead arsenate control was also less than normal for 1956 but not appreciably below that obtained in 1949. Parathion performed consistently throughout the period.

The results from the field tests on both broods show that TDE was very much less effective in 1956 than it had been in earlier years and that parathion and lead

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Table 1.—Red-banded leaf roller control, first brood, in experimental tests in western New York apple orchards.

Material and Pounds per 100 Gallons	Applied		Per Cent Reduction of Fruit Injury						
	Petal Fall	10-Day	1949[a]	1951	1952	1954	1955[b]	1956[a,b]	
50% TDE, 2		x	99	99				91	71
50% TDE, 1		x	96	98	99	100	93	77	56
50% TDE, 0.5		x	87	96				52	23
15% parathion, 2	x	x					99	99	82
15% parathion, 1	x	x	96	99					
15% parathion, 1		x				99			
Check (per cent fruits injured)			12	75	27	54	32	28	15

[a] Test in same block of apple trees on Sodus Fruit Farm in both 1949 and 1956.
[b] Test in adjacent blocks of apple trees on Kelley Farm in 1955 and 1956.

arsenate were of nearly the same efficacy throughout the entire period. This evidence would suggest that resistance of the red-banded leaf roller to TDE has developed to a measurable and serious degree in this area.

LABORATORY TESTS.—First brood egg masses were collected from the Kelley, Sodus Fruit Farm, and Chalupa orchards in the spring of 1956. Either 5 or 10 of the newly hatched larvae were placed on an apple leaf treated with various concentrations of TDE, parathion, or water check. Leaves were treated by dipping them into water suspensions of the materials. Just enough B-1956 was added to provide good wetting of the pubescent undersurface of the leaves. The treated leaves were dried and immediately placed in shell vials (9×2.5 mm.). Larvae were then added and the vials stoppered. The vials were kept in racks on a shelf in an 80° F. room where they were exposed to subdued daylight from a north exposure.

Results were determined by counting the live larvae after 48 hours, by which time most of the mortality due to the toxicants had occurred. Control was calculated by Abbott's (1925) formula and the results plotted on logarithmic-probability paper.

For the TDE tests, comparable series of vials were set up for all three strains on each day that tests were made. The supply of the Chalupa strain eggs was limited so fewer individuals of these were used in some setups. The TDE curves for the Kelley, Sodus Fruit Farm, and Chalupa strains are based on 1040, 1141 and 437 larvae, respectively. Only 120, 120 and 80 larvae were used, respectively, for parathion tests with the three strains so the results were not treated statistically and no exact comparisons are made.

Table 2.—Red-banded leaf roller control, second brood, in experimental tests in western New York apple orchards.

Material and Pounds per 100 Gallons	Number of Applications[a]	Per Cent Reduction of Feeding Areas				
		1948	1949	1951	1952	1956
50% TDE, 2	2	100				87
50% TDE, 1.5	2			99		
50% TDE, 1	2		97			
50% TDE, 4	1					82
50% TDE, 2	1		99			45
50% TDE, 1.5	1			99	98	
Lead arsenate, 3	2	94	80	90		76
15% parathion, 2	2					90
15% parathion, 1.5	2	96	82	87		
15% parathion, 1.5	1	92	72	87		
Check (injuries per 100 fruits)		34	21	54	59	63

[a] Timing for two applications has been about 1 week after beginning of hatch and again just after peak hatching. For one application the timing has been close to peak of egg hatching.

The dosage-mortality curves for TDE with the three strains of leaf roller are shown in figure 1. These have been calculated and analyzed by methods outlined by Bliss (1935). The curve for the Chalupa strain was found to be significantly different from the other two but no significant difference was found between the Kelley and the Sodus Fruit Farm strains. It is noted that the slope of the Chalupa strain is markedly steeper than for the others. According to Hoskins & Gordon (1956) changes in slope may be expected as resistance develops, due to a change in the heterogeneity of the population. It can be assumed then that the Chalupa strain is relatively homogeneous in its susceptibility to TDE, whereas in the other strains there is more heterogeneity due to a larger percentage of resistant individuals. As yet these populations have not become homogeneously resistant to TDE.

The LD 50's and LD 95's for the three strains are given in table 3 for TDE and for parathion. These show that for TDE the LD 95's for the Kelley and Sodus Fruit Farm strains are, respectively, 8.1 times and 9.9 times that for

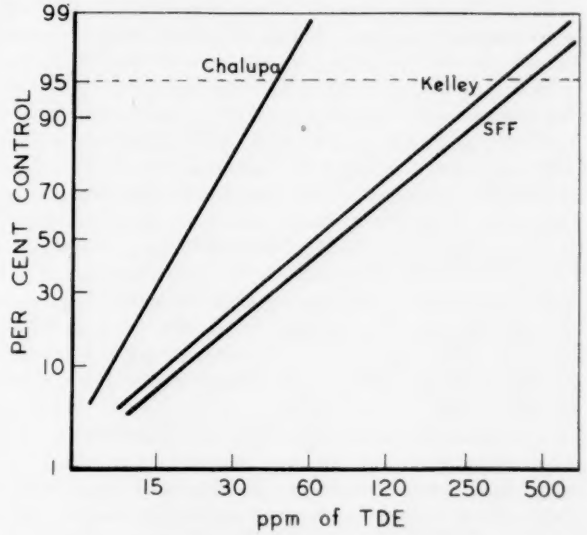

FIG. 1.—Dosage-mortality curves for red-banded leaf roller populations from three orchards.

Table 3.—LD 50 and LD 95 values for TDE and parathion in laboratory tests against three strains of red-banded leaf roller.

Leaf Roller Strain	P.P.M. TDE		P.P.M. Parathion[a]	
	LD 50	LD 95	LD 50	LD 95
Chalupa	19	43	19	24
Kelley	64	350	16	22
Sodus Fruit Farm	76	425	7	11

[a] Approximate values.

the Chalupa population. The data for parathion—for reason stated above—cannot be considered very conclusive. However, there is no indication that the Chalupa strain is more susceptible to parathion than the others. These data strongly indicate that the Kelley and Sodus Fruit Farm populations have developed a tolerance for TDE.

Summary and Conclusions.—The red-banded leaf roller was effectively controlled with TDE in all New York orchards from 1949 to 1954. The first indication of the development of TDE resistance was noted in 1954 and involved a second brood population in a single planting. The problem worsened in this planting in 1955 and poor results were reported with TDE in several other orchards. The list of "trouble orchards" was extended in 1956. However, the percentage of orchards exhibiting this condition is still small.

A study of the data from experimental field tests shows the results with TDE in 1955 and particularly in 1956 were not as satisfactory as those obtained in earlier tests. In one experiment where the standard New York recommendation was applied for second brood, control was less than 50% effective.

In laboratory studies conducted, leaf roller larvae were used from two orchards where TDE had been used since 1949 and from one planting where TDE had never been used. The dosage mortality curve for TDE for the last strain was steeper, these larvae being 8 to 9 times more susceptible to TDE than the other two. Response to parathion was similar for all three strains.

From grower experience and from field and laboratory tests it is concluded the red-banded leaf roller has developed a measurable degree of resistance to TDE in some New York apple orchards.

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Thrips Control on Cotton with Phosphorus Insecticides[1]

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Early-season damage to cotton by thrips (*Frankliniella* sp.) occurs in central Texas every year. Infestations usually are heavy when the weather is dry during late spring and early summer. Previous experiments w th chlorinated hydrocarbon insecticides in thrips control were reported by Ewing & Parencia (1949, 1950) and Parencia & Ewing (1951). Results obtained with these insecticides and a phosphorus compound, EPN, were reported by Parencia & Cowan (1953). Since that time, although more emphasis has been given to systemic insecticides employed as seed treatments for this control (Parencia *et al.* 1956), field tests have been conducted with conventionally applied new phosphorus insecticides as they have become available. It is the purpose of this paper to discuss thrips control obtained with phosphorus insecticides in comparison with the recommended chlorinated hydrocarbon insecticides in six field experiments conducted in 1954, 1955, and 1956 in the vicinity of Waco, Tex.

Procedure.—In experiments 1, 2, and 5 the plots were 16 rows wide and of sufficient length for an area of 0.2 acre. They were arranged in randomized blocks with four replications of treatments. Untreated plots were not included within the randomized-block experimental design, but were located in the same fields adjacent to the treated plots. In experiments 3, 4, and 6 the plots ranged from 2 to 5 acres in size, and there were no replications of treatments.

In the small-plot experiments emulsions were applied with a 4-row, tricycle-type, self-propelled machine constructed especially for spraying small areas; an engine mounted on the frame propelled the machine and drove the pump. The total volume of spray per acre was 2.5 gallons. In the large-plot experiments the emulsions were applied with 8-row, tractor-attachment, low pressure and low-volume spray machines at the rate of 2 gallons per acre. In all experiments one nozzle per row and a pressure of 60 pounds per square inch were used. Dusts were applied with rotary hand guns. Applications were usually made at weekly intervals.

The procedure and method of recording thrips data were the same as those reported by Parencia & Cowan (1953). Treatments and results are shown in table 1. Experiments 1 to 4 were considered as being completed at the end of the thrips-control period, and experiments 5 and 6 at the end of the growing season. Records pertaining to boll weevil (*Anthonomus grandis* Boh.) and cotton fleahopper (*Psallus seriatus* (Reut.)) infestations

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[2] In cooperation with the Texas Agricultural Experiment Station.

Table 1.—Effectiveness of several insecticides against thrips on cotton.

Insecticide (Pounds per Gallon in Sprays, Per Cent in Dusts)	Active Ingredient per Acre (Pounds)	Average Number of Thrips per Plant: Before Treatment	Average Number of Thrips per Plant: After Treatment
Experiment 1 (Spray applications June 8, 15, and 22, 1954)			
Toxaphene 6	0.75	7.0	4.0
Chlorthion 4	.25	10.0	7.4
	.375	7.4	6.1
Strobane 8	.75	7.7	5.6
Methyl parathion 2	.125	8.0	5.9
	.25	8.8	4.1
L.S.D. at 5% level	—	—	1.5
Untreated outside check	—	8.1	11.3
Experiment 2 (Applications June 2 and 11, 1955)			
Sprays			
Dieldrin 1.5	0.1	1.9	0.9
Guthion 1.8	.06	3.1	2.7
	.11	3.5	2.6
	.22	3.5	2.2
Dusts			
Guthion 2.5	.13	2.4	2.2
Endrin 2	.14	2.8	3.5
L.S.D. at 5% level	—	—	0.9
Untreated outside check	—	3.6	6.8
Experiment 3 (Spray application May 30, June 6 and 14, 1955)			
Toxaphene 6	0.75	6.8	3.4
Guthion 1.8	.06	4.0	4.7
	.11	5.4	3.9
Strobane 8	.75	4.4	3.3
Untreated check	—	4.2	6.4
Experiment 4 (Spray applications May 30, June 6 and 14, 1955)			
Guthion 1.8	0.11	14.2	7.0
Untreated check	—	14.2	10.2
Experiment 5 (Spray applications May 17, 24, 31, and June 7, 1956)			
Dieldrin 1.5	0.1	1.0	2.7
Guthion 2	.125	1.8	4.0
	.25	0.6	3.5
Dipterex[a]	.5	0.8	6.6
	1.0	1.1	5.9
Malathion 5	.31	0.7	6.3
L.S.D. at 5% level	—	—	1.4
Untreated outside check	—	0.8	8.4
Experiment 6 (Spray applications May 16 and 30, 1956)			
Dieldrin 1.5	0.1	0.4	2.8
Methyl parathion 2	.25	1.6	5.2
EPN 4	.25	1.1	3.0
Dipterex[a]	1.0	1.1	6.2
Guthion 2	.125	3.0	4.2
Untreated check	—	0.8	8.5

[a] 50% soluble powder.

and yields of seed cotton made in the last two experiments are given in table 2.

RESULTS.—In experiment 1 records made 24 hours after treatment (not shown in table) showed that both dosages of Chlorthion and the 0.25-pound dosage of methyl parathion gave initial kill equal to that obtained with toxaphene. In the seasonal average of records made 6 days after treatment, toxaphene gave significantly better

Table 2.—Effectiveness of several insecticides against the boll weevil and cotton fleahopper in thrips-control experiments in 1956.

Insecticide (Pounds per Gallon in Sprays, Per Cent in Dusts)	Active Ingredient per Acre (Pounds)	Per Cent of Weevil-Punctured Squares	Fleahoppers per 100 Terminals	Seed Cotton (Pounds per Acre): Total	Seed Cotton (Pounds per Acre): Gain Over Check
Experiment 5 (Spray applications May 17, 24, 31, and June 7)					
Dieldrin 1.5	0.1	12.1	7.0	492	146
Guthion 2	.125	16.7	11.5	432	86
	.25	12.2	10.6	509	163
Dipterex[a]	.5	12.6	10.8	406	60
	1.0	13.4	9.3	466	120
Malathion 5	.31	11.9	10.0	505	159
L.S.D. at 5% level	—	—	—	71	—
Outside check	—	26.2	15.9	346	—
Experiment 6 (Spray applications May 16, 30, and June 6)					
Dieldrin 1.5	0.13	11.7	8.6	743	397
Methyl parathion 2	.33	7.5	13.1	688	342
EPN 4	.33	4.8	11.4	604	258
Dipterex[a]	1.33	10.9	11.7	589	243
Guthion 2	.17	9.6	9.1	587	241
Untreated check	—	31.2	13.6	346	—

[a] 50% soluble powder

control than both dosages of Chlorthion or Strobane or the 0.125 pound dosage of methyl parathion.

In experiment 2 dieldrin gave significantly better control than any of the other insecticides. Guthion at the 0.22 pound spray dosage and as a dust was better than endrin dust.

In large plot experiment 3, toxaphene, Strobane, and the 0.11-pound dosage of Guthion were better than the 0.06-pound dosage of Guthion. Control with toxaphene was not so good as that usually obtained. Frequent rains occurred between treatments, and the period of residual control was probably reduced even though applications could be made at weekly intervals.

In large-plot experiment 4, in which the infestation was heavy, three spray applications of an 0.11-pound dosage of Guthion reduced the average infestation below that of the check by only 31.2%.

In experiment 5 dieldrin and both dosages of Guthion gave significantly better control than both dosages of Dipterex or malathion. There were no appreciable differences in boll weevil and cotton fleahopper infestations, but in all the treated plots they were considerably lower than in the outside check. In yields of seed cotton dieldrin, malathion, and the 0.25-pound dosage of Guthion were significantly better than the 0.5-pound dosage of Dipterex. Each treatment except 0.5 pound of Dipterex and 0.125 pound of Guthion gave good increases in yield over the outside check.

In large-plot experiment 6 there was no difference in control of thrips between dieldrin at 0.1 pound and EPN at 0.25 pound per acre. Guthion at 0.125 pound was considerably less effective, and poor control was obtained with methyl parathion at 0.25 pound and with Dipterex at 1 pound. Thrips dosages used in the first two applications were doubled in the third application for control of overwintered boll weevils. Boll weevil infestations were reduced considerably by all insecticides. Dieldrin produced the highest increase in yield, followed by methyl parathion, with small differences among the other three insecticides.

CONCLUSIONS.—Control of thrips with such chlorinated hydrocarbon insecticides as dieldrin and toxaphene was

better than that obtained with the phosphorus compounds Guthion, methyl parathion, and Dipterex. The last material gave very poor control. It appears that the period of residual control afforded by phosphorus insecticides is shorter than that afforded by chlorinated hydrocarbon insecticides, especially when low dosages are used.

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Influence of Surface Color on Mosquito Landing Rates[1]

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In the course of studies of mosquito attractants, chemicals that stained the white test surfaces appeared to be attractive and it was necessary to select a color for the test surface that would not show this effect. Experiments were therefore conducted in which disks of various shades and colors were placed in cages with mosquitoes to find the shade or color that would give the largest landing rates.

Brown (1954) showed that four Canadian species of *Aedes* preferred to land on dark cloths; black, dark blue, and red were attractive, whereas white, green, and yellow were unattractive. A negative correlation between landing rates and reflectivity was found in the wave length range between 475 and 625 mμ. From later work Brown (1955) concluded that light-colored clothing did not increase the attack of mosquitoes on the face, and significantly more landings were received with black clothing than with white.

Materials and Equipment.—Food coloring was used for the red, yellow, and blue; clothing dye was used for the black. The secondary colors were made by mixing each pair of primary colors in equal proportions. Water solutions of each color were prepared at various concentrations. White filter-paper disks 12.5 cm. in diameter were submerged in a solution of the dye for 1 minute, the excess material was drained off, and the disks were air-dried. The disks were trimmed to 10-cm. diameter to remove a dark border.

The light reflected by each colored disk was measured with an exposure meter (General Electric Model No. DW68). The meter, a microscope light (Spencer Model No. 370), and a circular stage were mounted on a ringstand. This apparatus was used in a dark room. A white disk was placed on the stage and the equipment adjusted to record 70-foot-candles on the meter. The light reflected by each colored disk in the same position as the white disk is presented in table 1.

The tests were conducted with caged adult mosquitoes—*Aedes aegypti* (L.) reared in the laboratory and *A. taeniorhynchus* (Wied.) and *A. sollicitans* (Wlk.) from larvae and pupae collected from marshes on the Florida east coast. Each test cage contained from 1,500 to 2,000 mosquitoes of both sexes. The cages (18 inches square) have screened sides and back, a cloth sleeve front, a plywood top, and a glass bottom. To facilitate counting, the cage was placed on a box equipped with a 75-watt light bulb controlled by a switch.

Test Methods.—Two disks of different shades or colors were hung firmly about 3 inches from either side and 6 inches from the top of the cage. The mosquitoes were disturbed, and after 30 seconds the number resting on the disks was recorded. The position of the disks was reversed and a second count taken. The average landing rates were used in the statistical analysis. Turning on the light at the bottom of the cage during the counting period did not disturb the mosquitoes on the disks. All tests were run in a fluorescent-lighted room.

Table 1.—Foot-candles of reflected light of colored disks. (Average of 2 readings.)

Concentration of Dye (Per Cent)	Red	Yellow	Blue	Black	Orange	Violet	Green
100	15	38.5	4.5	5	17	4.5	4.5
80	16.5	41	4.5	5	20	5	5.5
60	19	42	6.5	6	21	6	6.5
40	22	46.5	9	7	24	8	9
20	26.5	48	14	9	28	10	13
10	31.5	50.5	19	15	32	14.5	18
5	38	52.5	25	24	37.5	20	25
2.5	43	58	32	37	42.5	28	33
1.0	51.5	61	41	53	51.5	40	46

In all experiments a balanced, incomplete-block design was employed. With this design each color or shade was paired against each other color or shade in the experiment. White disks were included as standards of comparison. An adjusted average landing rate, which compensates for

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[2] The authors acknowledge the assistance of Carroll N. Smith, under whose supervision this work was done. R. L. Fye, Nelson Smith, R. A. Sutton, and D. W. Meifert took an active part in the testing.

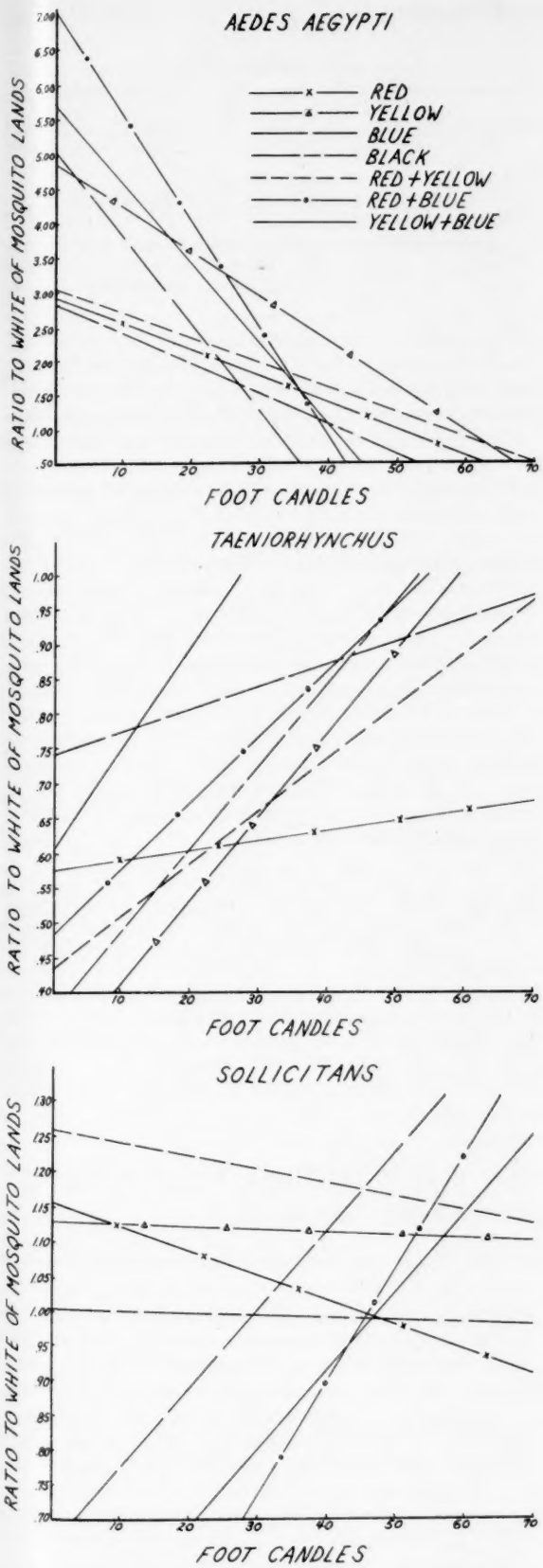

FIG. 1.—Landing rates of mosquitoes on different shades of color.

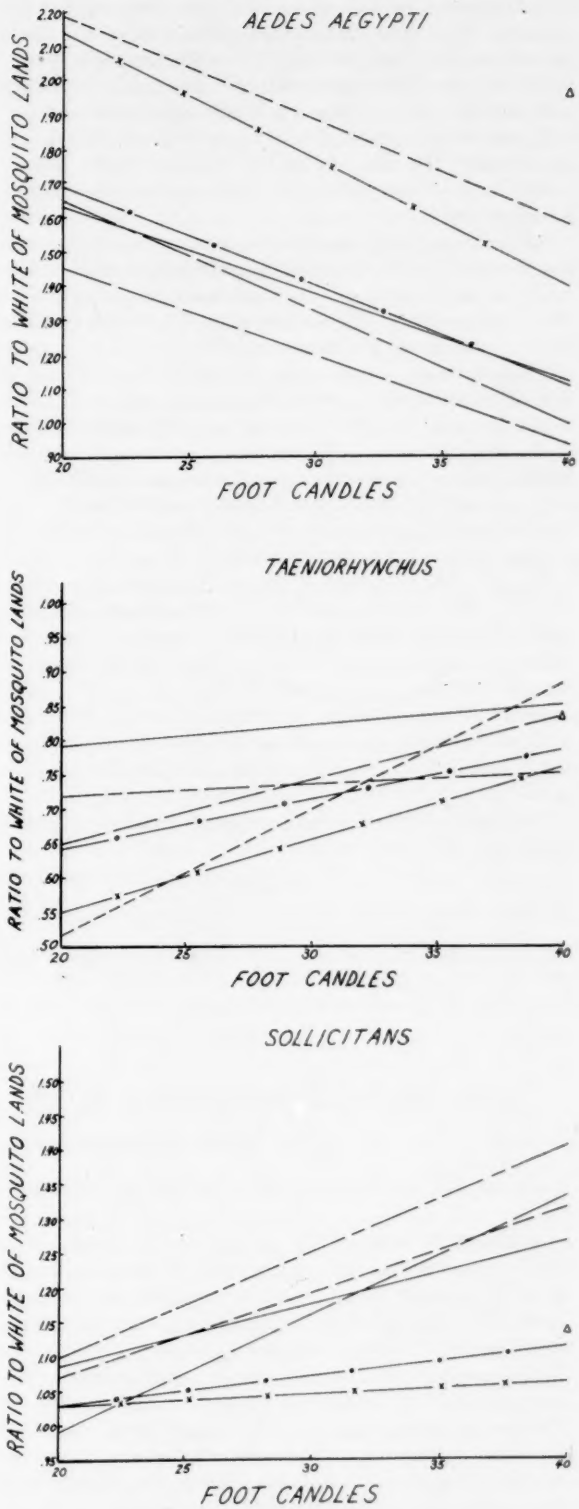

FIG. 2.—Landing rates of mosquitoes on colors of equal brightness

variations of the mosquitoes and testing conditions, was computed by the statistical method (Kempthorn 1952) suggested by J. U. McGuire. The differences in the number of mosquitoes landing on two disks required for significance (5%) were determined. The ratio of the landing rate on the white disk to that on the various colors or shades of colors was computed, and regression lines were calculated of foot-candles of reflected light over ratio.

TESTS WITH VARIOUS SHADES OF COLOR.—Duplicate experiments (18 tests) with the various shades of any one color were conducted with each species. The results are presented in figure 1.

Against *aegypti* the darker shades of any one color were progressively more attractive than the lighter shades. The ratios of landings on the darkest shade of each color to white ranged from 2.9 for black to 7.7 for violet. The landing rate of *taeniorhynchus* was the reverse of *aegypti*, the lighest shade of each color having the largest landing rate. The corresponding ratios ranged from 0.4 for black to 0.7 for blue. *A. sollicitans* preferred the darker shades of the brighter colors—yellow, red, and orange—and the lighter shades of the colors containing blue. Little preference was shown with the different shades of black. The ratio of the darkest shade of each color to white ranged from 0.3 for violet to 1.2 for orange.

TESTS WITH COLORS OF EQUAL BRIGHTNESS.—Disks were treated with predetermined concentrations of each color producing about 20 and 40 foot-candles of reflected light. Yellow disks could not be made dark enough to obtain a 20-foot-candle reading. Seven colors with light readings of about 40-foot candles and white were tested against the three species of mosquitoes. Only six colors were included at 20 foot-candles. The results are presented in figure 2.

The various colors in shades of equal brightness showed significant differences as follows:

Aedes aegypti

40 foot-candles: Yellow > orange and red > green, violet, black, blue, and white.

20 foot-candles: Orange and red > violet, black, green, and blue > white.

Aedes taeniorhynchus

40 foot-candles: White > orange, green, yellow, and blue > violet, red, and black.

20 foot-candles: White > green and black > blue and violet > red and orange.

Aedes sollicitans

40 foot-candles: Black, blue, orange, and green > yellow, violet, red, and white.

20 foot-candles: No significant difference in color or white. The descending order of attractiveness was as follows: Black, green, orange, violet, red, and blue.

Ultraviolet, visible, and near infrared spectral directional reflectance curves have been obtained for the disks that reflected 40 foot-candles. There was a negative correlation between the landing rates and the amount of light reflected in the range of 475 to 525 mμ wave length with *aegypti* but not with the other species.

SUMMARY.—Tests comparing landing rates on colored paper disks were conducted with *Aedes aegypti* (L.), *A. taeniorhynchus* (Wied.), and *A. sollicitans* (Wlk.). The disks were treated with dyes of the primary and secondary colors in a range of shades. The darker shades of each color attracted the most *aegypti*, while the lighter shades attracted the most *taeniorhynchus*. The darker shades of the brighter colors and the lighter shades of the colors containing blue were most attractive to *sollicitans*.

With disks reflecting about 40 foot-candles of light, the attractiveness of the colors was in this order: For *aegypti*, yellow, orange, red, green, violet, black, and blue; for *taeniorhynchus*, orange, green, yellow, blue, violet, red, and black; for *sollicitans*, black, blue, orange, green, yellow, violet, and red.

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Comparative Effectiveness of Malathion and Malathion-Yeast Hydrolysate Bait Sprays for Control of the Melon Fly[1]

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Attempts to control the melon fly, *Dacus cucurbitae* Coq., by use of bait sprays have not yielded consistently effective control. As early as 1910, Marsh attempted to control this tephritid fly on cucumbers and cantaloupes with aqueous sprays containing molasses and either Paris green or lead arsenate. Similar attempts were later made in 1914 by Severin *et al.* and in 1917 by Back & Pemberton using sprays containing brown sugar and arsenate of lead or sodium arsenite. In all of these reports the authors stated that, although the application of bait sprays caused a reduction in the number of flies, no practical control was obtained. Holdaway (1945) found that bait sprays containing tartar emetic failed to give consistent control and thus could not be recommended for melon fly control. Recently, however, Steiner (1954, 1955) reported that effective control of fruit flies, including the melon fly, was obtained by use of a bait spray containing yeast hydrolysate and either malathion or parathion. According to Steiner (1954), this bait spray is both attractive and toxic to the melon fly, and is effec-

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tive in controlling this pest attacking passion fruits (*Passiflora edulis* f. *flavicarpa* Degener). However, he stated that the use of this spray for the control of the melon fly attacking other crops had not been fully investigated.

During the past year some aspects on the use of malathion-yeast hydrolysate bait sprays for melon fly control were investigated. Among these investigations were those which were conducted to determine whether the addition of yeast hydrolysate increased the effectiveness of malathion sprays. This paper presents the results of these studies in order to call attention to some of the problems that have incurred.

GENERAL PROCEDURE.—The materials used in these studies were malathion, 25% wettable powder, and yeast hydrolysate, a water soluble proteinaceous food supplement which was found by Hagen & Finney (1951) to increase the fecundity of fruit flies. These materials were used as sprays either alone or in combination. The concentration of each ingredient in the sprays is expressed in terms of the weight in pounds of the commercial product per 100 gallons of water.

Residual effects of malathion and malathion-yeast hydrolysate sprays.—Studies on the residual effects of malathion and malathion-yeast hydrolysate sprays were conducted in weedy abandoned cucumber and watermelon fields in which considerable numbers of adults were present on various weeds, such as: fuzzy rattle pod, *Crotolaria incana* L.; wild euphorbia, *Euphorbia geniculata* Ortega; cocklebur, *Zanthium saccharatum* Wall.; and spiny amaranth, *Amaranthus spinosus* L. These plants as well as others were found to be highly attractive to the adult melon flies (Nishida & Bess 1957). Individual plants or groups of two plants were tagged and all debris, rocks, and large lumps of soil were removed from the surface of the ground beneath the plants. Dieldrin and DDT sprays were applied to the soil to eliminate ants, which often carry away dead flies that drop to the ground. Each plant was shaken briskly several times prior to spraying to drive away flies that were on the plants, and then approximately 350 cc. of spray was applied per plant using a 3-gallon capacity compressed-air sprayer which delivered coarse droplets. The sprays were various concentrations of malathion and malathion plus yeast hydrolysate with three replications per treatment. After treatment, records were taken at hourly intervals on the number of dead flies on the ground. At the end of the observational period a spray containing 2 pounds malathion and 1 pound yeast hydrolysate was applied to the experimental plants in order to determine whether flies were still present in the immediate vicinity.

Effectiveness of malathion and malathion-yeast hydrolysate sprays on control.—Experiments to determine the effectiveness of malathion and malathion-yeast hydrolysate sprays for melon fly control were conducted on the University of Hawaii campus in Manoa Valley and at the sub-station at Waimanalo. On the campus there were five cucumber plots which ranged in size from 50×90 feet to 50×150 feet with each plot surrounded on all sides by 4 to 8 rows of corn. Three plots were treated with 2 pounds malathion plus 1 pound of yeast hydrolysate per 100 gallons. The remaining two plots were sprayed with malathion alone at the rate of 2 pounds per 100 gallons. Although there were three replications in the bait spray treatment, data obtained from one were later discarded because of poor fruit yields caused by a very high incidence of mosaic. The cantaloupes at Waimanalo were planted in a 85×486-foot area in which two rows of corn were planted along the two sides, but not along the ends. This field was divided into four 85×120-foot plots. The two treatments, malathion at a concentration of 2 pounds per 100 gallons and malathion at the concentration of 2 pounds plus 1 pound yeast hydrolysate per 100 gallons, were assigned at random to these four plots.

In both the cucumber and cantaloupe experiments, the methods of application and frequency of application were similar. The applications were made only to corn plants, for it has been shown that melon fly adults are found in greatest numbers on favorable non-host plants rather than on the crop to be protected (Nishida & Bess 1950, Nishida 1954, Nishida & Bess 1957). The corn plants were thoroughly sprayed using conventional type sprayers at a pressure of approximately 300 p.s.i. at intervals of once to twice a week.

The effectiveness of the treatments were evaluated on the basis of records taken on both melon fly abundance and fruit infestation. Melon fly abundance was determined by counting the number of adults on corn plants. In determining fruit damage, cucumber and cantaloupe fruits were examined in the field and the number of stung and unstung fruits recorded. Records were taken on cucumber fruits ranging from 0.5 to 8 inches in length and on cantaloupe fruits approximately 0.75 to 5 inches in length. All fruits with oviposition scars were counted as infested regardless of whether eggs or maggots were present within the fruit or not. On each sampling date, eight samples were taken from different parts of each cucumber plot, and six from each cantaloupe plot.

RESULTS.—*The residual effects of malathion and malathion-yeast hydrolysate sprays.* The data presented in figure 1 show the comparative residual kill between malathion and malathion-yeast hydrolysate sprays when different concentrations were applied to fuzzy rattle pod, *Crotolaria incana* L., and cocklebur, *Xanthium saccharatum* Wall. The kill obtained was consistently higher with sprays containing malathion and yeast hydrolysate than those containing malathion alone. It is also evident, that, regardless of the concentrations tested, the kill was highest immediately after the spray application and that it decreased rapidly so that by the third day after application the kill was exceedingly low as compared with the initial kill. This rapid decrease in kill was evidently not due to the depletion of flies in the immediate vicinity, for when a fresh bait spray was applied the kill increased to a level comparable to that obtained initially.

Because these results indicated a rapid loss of effectiveness with time, an experiment was conducted to determine whether the loss of effectiveness was due to the loss of effectiveness of the toxicant or the bait. It was reasoned that if the toxicant had deteriorated before the bait, then there should be no kill when additional yeast hydrolysate was applied. However, if the toxicant was still effective, the application of bait alone should kill the adults feeding on the bait. Accordingly, cocklebur plants were sprayed with (1) 2 pounds of malathion plus 1 pound of yeast hydrolysate per 100 gallons and (2) 4 pounds of

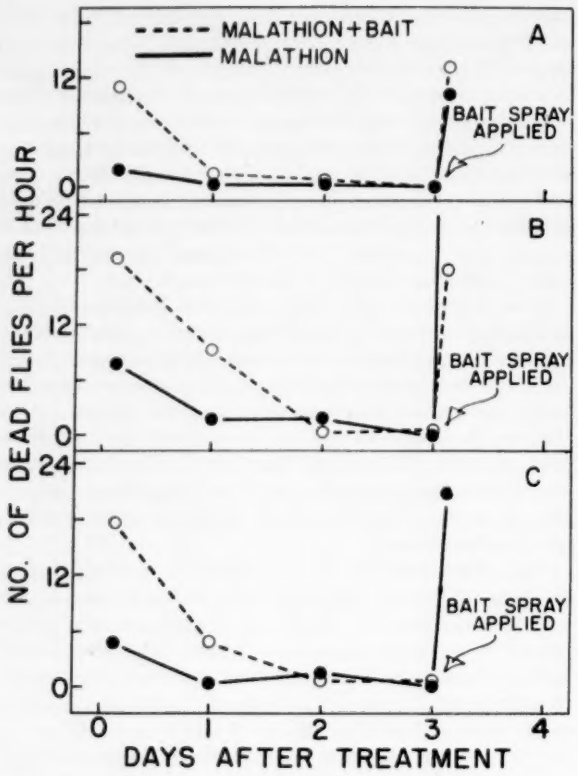

FIG. 1.—Comparative residual kill among various concentrations of malathion-yeast hydrolysate and malathion sprays: *A*, comparison between 2 pounds malathion plus 1 pound yeast hydrolysate and 2 pounds malathion per 100 gallons; *B*, comparison between 4 pounds malathion plus 2 pounds yeast hydrolysate and 4 pounds malathion per 100 gallons; *C*, comparison between 8 pounds malathion plus 4 pounds yeast hydrolysate and 8 pounds malathion per 100 gallons. The final bait spray applied on the third day contained 2 pounds malathion plus 1 pound yeast hydrolysate per 100 gallons.

malathion plus 2 pounds of yeast hydrolysate per 100 gallons. After 3 days, when there was a considerable decrease in kill, all plants were sprayed with yeast hydrolysate alone at a rate of 1 pound per 100 gallons. On the fourth day all plants were sprayed with 2 pounds of malathion plus 1 pound of yeast hydrolysate.

The data obtained, presented in figure 2, indicate that as in the previous experiments the rate of kill declined rapidly. However, when the bait alone was applied there was an increase in kill which showed that the malathion sprayed initially was still toxic, and hence, the rapid decline in kill of the bait sprays was related to the loss of attractiveness and/or palatability of the bait. That the bait was no longer attractive was observed when counts on dead flies were being made. Many flies were seen on the untreated plants, some of them as close as a foot away from the treated ones, evidently not attracted to the bait. Furthermore, there was considerable increase in kill when the final application of the toxicant plus bait was made on the fourth day, which also indicated that there were many adults in the immedate vicinity.

Following this experiment, the effect of increased concentrations of yeast hydrolysate in prolonging the period

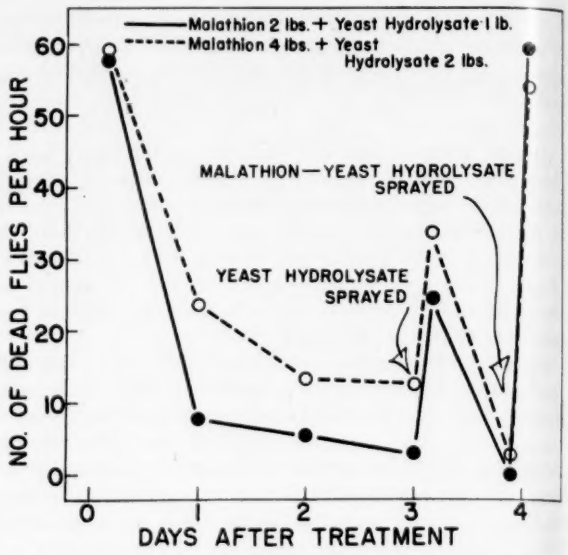
Malathion 2 lbs. + Yeast Hydrolysate 1 lb.
Malathion 4 lbs. + Yeast Hydrolysate 2 lbs.
MALATHION—YEAST HYDROLYSATE SPRAYED
YEAST HYDROLYSATE SPRAYED
NO. OF DEAD FLIES PER HOUR
DAYS AFTER TREATMENT

FIG. 2. Comparison between the rapidity of loss in effectiveness of yeast hydrolysate plus malathion with malathion alone. The spray applied on the third day contained 1 pound yeast hydrolysate per 100 gallons of water, and the malathion-yeast hydrolysate spray applied on the fourth day contained 2 pounds of malathion and 1 pound yeast hydrolysate per 100 gallons.

of effectiveness of the bait sprays was also studied. Bait sprays containing 2 pounds of malathion, and 1, 2 or 4 pounds of yeast hydrolysate per 100 gallons were applied to wild euphorbia, *Euphorbia geniculata* Ortega. The results presented in figure 3 indicate that, although there was a higher kill with the high concentrations of yeast hydrolysate, the differences were not so great as might be desired from a practical standpoint.

Effect of malathion and malathion-yeast hydrolysate sprays on control. Comparative data on the effects of malathion and malathion-yeast hydrolysate sprays on melon fly population and cucumber fruit infestation obtained

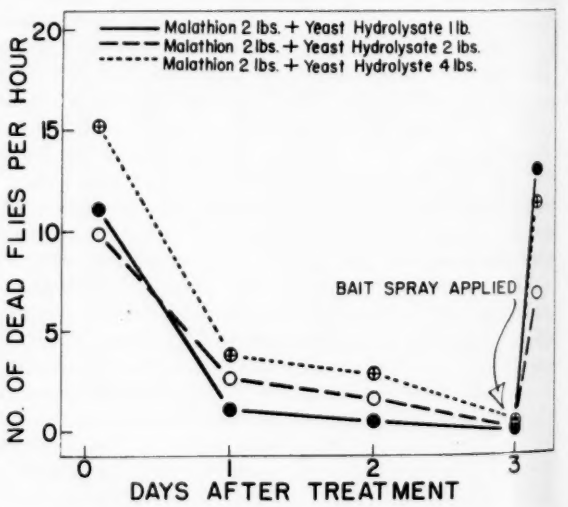
Malathion 2 lbs. + Yeast Hydrolysate 1 lb.
Malathion 2 lbs. + Yeast Hydrolysate 2 lbs.
Malathion 2 lbs. + Yeast Hydrolyste 4 lbs.
BAIT SPRAY APPLIED
NO. OF DEAD FLIES PER HOUR
DAYS AFTER TREATMENT

FIG. 3.—The effect of increasing the concentrations of yeast hydrolysate on the effectiveness of malathion-yeast hydrolysate spray.

from the experiment conducted at Manoa, presented in figure 4 and table 1, indicate that following the first application there was a consistent and marked decline in fly population in both the malathion and malathion-yeast hydrolysate treated plots. Furthermore, the fly population in the non-baited and baited plots fluctuated in a very similar manner, and there was no significant difference in the fly population between the two plots during the treatment period. Data on the effect of the treatments on cucumber fruit infestation, presented in table 1, also indicate that there was no significant difference in the percentage of stung fruits between the malathion and malathion-yeast hydrolysate treated plots. This lack of difference in infestation is not surprising in view of the lack of differences in the fly population between the baited and non-baited plots during the fruiting period.

Comparative data on the effects of malathion and malathion-yeast hydrolysate sprays on fly population and cantaloupe fruit infestation obtained from the experiment conducted at Waimanalo, presented in figure 4 and table 1, also indicate that, as in the case of the cucumber experiment, the fly population declined markedly after the first application and that the mode of fluctuation of the fly populations in the baited and non-baited plots were remarkably similar. The fruit infestation data also showed no significant difference in the percentage of infested fruits between the baited and non-baited plots (table 1).

DISCUSSION.—The results obtained from this study showed that (1) when baited and non-baited malathion sprays were applied residually to individual plants favored by adult melon flies, the baited spray gave a higher kill than the non-baited one and (2) when the same sprays were applied to corn planted around cucumber and cantaloupe fields, there were no significant differences in either fly population or fruit infestation between plots sprayed with baited and non-baited sprays. These results

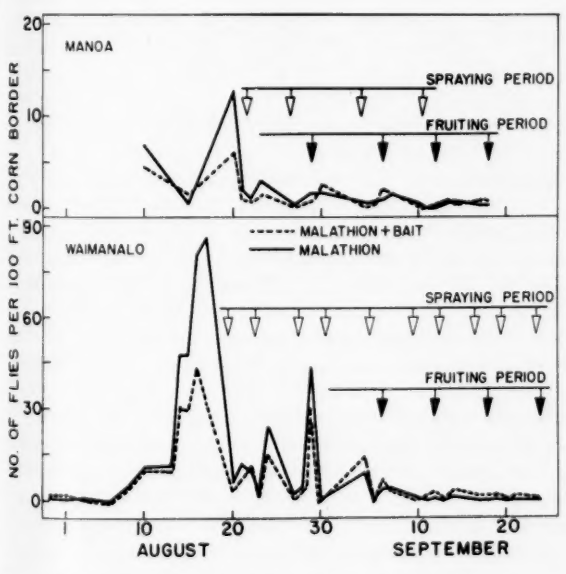

FIG. 4. Comparative data showing melon fly population trends in plots treated with malathion and malathion-yeast hydrolysate sprays at the Manoa campus and Waimanalo. Light arrows indicate spray dates; dark arrows, fruit sampling dates.

Table 1.—Comparative percentages of infested cucumber and cantaloupe fruits in plots sprayed with malathion-yeast hydrolysate bait spray and malathion alone, 1956.

SAMPLING DATES	OBSERVED INFESTATION: Baited Plots — Total Fruits	Baited Plots — Mean Per Cent Infestation[a]	Non-Baited Plots — Total Fruits	Non-Baited Plots — Mean Per Cent Infestation[a]
Cucumber (Manoa Campus)				
Aug. 29	161	18.6	216	22.1
Sept. 6	471	31.8	452	32.5
Sept. 12	569	23.5	515	19.1
Sept. 18	326	42.3	257	40.4
Cantaloupe (Waimanalo)				
Sept. 6	865	17.4	428	11.6
Sept. 12	1328	19.0	669	18.8
Sept. 18	630	28.3	315	24.4
Sept. 25	671	13.8	533	15.9

[a] Percentages were transformed to angles in the analysis of variance (Snedecor, 1956) and the calculated F values all fell below the tabular F values required for significance at the 5% level.

show that data obtained from isolated plant treatments and those obtained from actual control experiments are not correlated. This lack of correlation, evidently observed by early workers (Marsh 1910, Severin *et al.* 1914, Back & Pemberton 1917), indicate that the evaluation of the effectiveness of bait sprays as a control measure cannot be based entirely on data obtained by spot treating individual plants.

The cause of the lack of agreement in the results obtained from these two types of experiments is not clearly understood. Such results might be accounted for by differences in the method of application and the resulting differences in the manner in which flies were killed. In the experiment in which individual plants were treated, all flies were first driven off the plant prior to treatment, and the applications made by means of a knapsack sprayer. However, in the experiment in which the corn plants harboring flies were sprayed, the flies were not driven off the plant prior to spraying, and the applications were made by use of power equipment at 300 p.s.i. In the former experiment, the kill is evidently due to flies returning to the treated plants and thereby coming in contact with the treated surface. In such a situation the kill with the baited malathion spray would be higher than with the non-baited one because of the added kill resulting from feeding. However, in the latter experiment, the kill resulting from direct contact apparently accounted for much of the mortality, for counts made soon after spraying showed a 100% reduction of flies with both the baited and non-baited malathion sprays. In other words, the effect of the bait *per se* was completely masked by the high kill that resulted from direct space action.

The reason why there were no significant differences in either fly population or fruit infestation between plots treated with malathion and malathion-yeast hydrolysate sprays is also not clearly understood. It may be attributed to the high immediate kill obtained with both baited and non-baited sprays. Because of the immediate kill there was a simultaneous reduction of flies in both

baited and non-baited plots soon after treatment. However, on account of the relatively short period of effectiveness of the residues of both types of sprays, the populations rose simultaneously 1 to 2 days after treatment. Following each successive application the fly populations were reduced to the same extent with both sprays. Hence the effect was essentially the same as when all plots were treated with a non-baited malathion spray.

Interplot movements of flies were apparently not important in this study because there were no untreated plots from which flies could move to the baited plots. The movements involved were largely those from the outside, untreated, surrounding areas. From such untreated areas flies may have moved to the baited plots in larger numbers than to the non-baited plots; however, considering the same rate of fly increase in the respective plots, this type of movement cannot account adequately for the results obtained.

SUMMARY.—Experiments to determine the effectiveness of malathion and malathion-yeast hydrolysate bait sprays in controlling the melon fly, *Dacus cucubitae* Coq., were conducted during 1956. As a residual spray applied to isolated plants the baited spray gave a higher kill than the non-baited. The period of residual effect of both baited and non-baited sprays was short, approximately 1 to 2 days. Although in residual tests conducted by spraying isolated plants the baited spray gave a higher kill than the non-baited, in actual field tests on the control of the melon fly attacking cucumbers and cantaloupes there were no significant differences in fly population and fruit infestation between baited and non-baited plots. The lack of correlation between the results obtained in these tests and the failure to obtain significant differences in control between baited and non-baited plots need further investigation before final conclusions can be reached; however, the results of this study indicate the need for actual control experiments in evaluating the effectiveness of baits against the melon fly.

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The Absorption, Metabolism, and Excretion of C^{14}-Labeled Allethrin By House Flies[1]

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The mode of action and metabolism of pyrethrins in insects has been investigated by several workers and is extensively reviewed by Metcalf (1955). The recent synthesis of radioactive allethrin by Acree *et al.* (1954) and Winteringham *et al.* (1955) has added further means for studying the metabolism of the pyrethroid insecticides. Winteringham *et al.* (1955), working with randomly labeled C^{14}-pyrethrins and C^{14}-allethrin labeled on the allthrolone portion of the molecule, have studied their absorption and metabolism in adult house flies (*Musca domestica* L.). Their findings demonstrate that the pyrethroid esters are readily absorbed by insects and metabolized into significant amounts of relatively noninsecticidal substances in 24 hours. The addition of the synergist piperonyl cyclonene reduced both the rate of absorption of the pyrethroids and their metabolism *in vivo*.

In the experiments reported in this paper, C^{14}-allethrin, labeled on the chrysanthemum monocarboxylic acid moiety, was applied alone and in combination with the synergist piperonyl butoxide to female house flies to study the processes of absorption, metabolism, and excretion.

MATERIALS AND METHODS.—*Experimental Compounds.*—The *dl-cis*, trans-C^{14}-allethrin (*dl*-allethrolone ester of *dl-cis, trans*-2-C^{14}-chrysanthemum monocarboxylic acid) was synthesized and purified by Acree *et al.* (1954) and had an observable specific activity of 300 counts per minute (corr.) per microgram when assayed as "weightless" samples in a windowless gas flow proportional counter. At the time of purification the C^{14}-allethrin was estimated to be approximately 98% pure, as indicated by ultraviolet absorption curves. However, subsequent analyses by several paper chromatographic systems demonstrated the presence of C^{14}-labeled impurities (Acree & Babers 1956).

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[2] The authors express their gratitude to Fred Acree, Jr., and Frank H. Babers of the Entomology Research Division for making available the labeled allethrin used in these studies.

The sample of C14-allethrin received was found to have a radiochemical purity of 75%, when analyzed by paper chromatographic methods throughout the study (mean of 20 determinations). Nearly 5% of the total radioactivity ran with the Rf of chrysanthemum monocarboxylic acid by normal phase methods, and 20% behaved as an unknown contaminant. Because of the importance of the radiochemical impurities present in the sample, care was taken to determine the actual amount of these substances absorbed by the flies, as will be explained under "Results." The piperonyl butoxide used in the synergist studies was a purified sample. Nonradioactive technical allethrin, chrysanthemum monocarboxylic acid, and allethrolone were employed as chromatographic standards.

Methods of Analysis.—Radiometric assays were made with a windowless gas-flow proportional counter and a conventional scaler. All samples were prepared in triplicate in aluminum planchets, counted for a sufficient length of time to attain a standard error of ±5% (Calvin *et al.* 1949), and the total activity was computed from the mean corrected counting rates. To obviate losses due to the volatility of allethrin or related compounds, samples were counted immediately after preparation.

Paper chromatographic techniques used for the separation and detection of allethrin and its possible degradation products were essentially those described by Winteringham (1952). The reverse-phase method was modified to give greater separation of allethrin (Rf 0.25) and its hydrolysis products (Rf 0.84) by impregnating Whatman No. 4 paper with 3% of petroleum jelly in chloroform. An additional method was adopted for the separation of allethrin (Rf 0.93), chrysanthemum monocarboxylic acid (Rf 0.40), and allethrolone (Rf 0.05), and is a modification of a system reported by Mitchell (1954) for DDT and related compounds. Strips of Whatman No. 1 paper were impregnated with a 50% solution of N,N-dimethylformamide in C.P. acetone (v/v) and were dried for 5 minutes at room temperature. The mobile phase consisted of Shell Rubber Solvent (technically pure nonaromatic hydrocarbons, b.p. 80°–102° C.) saturated with dimethylformamide. Good separation of the compounds was obtained with a 20-cm. ascending development.

Paper chromatograms were analyzed radiometrically by cutting the strips into 1-cm. sections for use in the windowless counter. Nonradioactive standards were added to the chromatograms prior to development for color detection, and losses due to volatility were negligible on impregnated strips. All chromatograms were developed at a temperature of 24° to 25° C.

Experimental Procedures.—The insects used in these studies were laboratory-reared 3- to 5-day-old female house flies of a DDT-resistant strain designated as Orlando No. 1. For quantitative studies of the absorption and excretion of C14-allethrin, a system was designed for immobilizing house flies to eliminate the losses and gains due to "rub off," cross-contamination between flies, and ingestion of contaminated excreta that are possible when flies are allowed the freedom of a cage.

Strips of Scotch pressure sensitive masking tape cut to 2 mm. in width were attached to 6-inch squares of window glass, four equally spaced across each plate. House flies, anesthetized with carbon dioxide, were attached approximately 6 mm. apart by the sterna to the tape on each plate with small drops of quick-drying Duco cement. Food was furnished throughout the experimental period by placing cotton strings soaked in a 5% sucrose solution in front of the flies. Excreta were quantitatively collected by placing strips of Whatman No. 1 filter paper 5 mm. wide under the posterior abdominal regions of the attached flies.

For treatment the immobilized flies were lightly anesthetized with carbon dioxide. Acetone (C.P.) solutions of the toxicants were applied to the dorsal thoracic surfaces with a micropipette, each fly receiving 0.4±0.02 microgram of C14-allethrin, alone or with piperonyl butoxide at a 1:1 ratio in 1 microliter of the solvent.

Absorption of C14-allethrin was determined at intervals of 4, 8, 12, and 24 hours. Groups of 50 flies were anesthetized and removed from the glass plates by passing a sharp scalpel between the tape and sterna. The flies were then carefully placed in a glass funnel screened at the apex and rinsed with ten 10-ml. aliquots of acetone. The rinses were concentrated under a gentle air stream and evaluated radiometrically and chromatographically. To check recoveries and the nature of the radioactivity remaining in the flies, groups of the rinsed flies were macerated with sand in a mortar and extracted successively with *n*-hexane and acetone containing 0.1% acetic acid. The extracts were concentrated and analyzed as previously described.

The excretion of C14-allethrin or its metabolites was similarly investigated by removing the filter-paper strips containing the deposited excreta at the same intervals, each time replacing them with new strips. The feces were then extracted from the paper with dilute acetic acid and aqueous acetone, and the extracts concentrated for radioassay and chromatography. All experiments were conducted at a temperature of 24° to 25° C.

RESULTS.—The knock-down and mortality of the flies were investigated by treating groups of 50 with radioactive allethrin, alone and in combination with piperonyl butoxide, and observing their actions in screen-top pint jars for intervals up to 24 hours (table 1). Paralysis of flies treated with the synergist combination was initially slower than that induced by allethrin alone, but it eventually increased to double that of the latter when the maximum knock-down occurred at 2 hours. Allethrin alone and applied jointly with the synergist caused 2 and 12% mortality, respectively, at 24 hours, whereas the mortalities of immobilized flies treated similarly were 2 and 13%. Mortality of the flies in the controls did not exceed 2% in any of the tests.

Absorption.—Allethrin rapidly penetrated the integument of the house flies, 46% of the applied dose being absorbed in 4 hours (table 2). The process continued at a

Table 1.—Toxicity of C14-allethrin alone and with piperonyl butoxide to female house flies.[a]

C14-ALLETHRIN	PER CENT KNOCK-DOWN AFTER (HOURS)						PER CENT MORTALITY AFTER 24 HOURS
	½	1	2	4	8	12	
Alone	14	24	31	7	2	2	2
Plus piperonyl butoxide	8	21	62	56	18	16	12

[a] Mean of two or three tests.

Table 2.—Per cent adsorption of total dose and excretion of absorbed dose by female house flies treated topically with C^{14}-allethrin alone and with piperonyl butoxide.[a]

Hours	Allethrin		Allethrin Plus Piperonyl Butoxide (1:1)	
	Absorption	Excretion	Absorption	Excretion
Live flies				
4	46.1	6.5	40.5	4.7
8	53.9	20.6	50.3	13.5
12	71.3	27.3	62.0	20.8
24	76.3	44.4	65.0	36.4
Cyanide-killed flies				
24	77.5	—	73.5	—

[a] Total dose represents about 75% pure ester. (Mean of 2 or 3 tests.)

decreased rate until at the end of 24 hours approximately 76% of the total dose had been absorbed. Piperonyl butoxide applied jointly at a 1:1 ratio appeared to decrease the rate of penetration of allethrin, with a maximum reduction of 13% at 12 hours but less than 2% at the end of 24 hours. When flies were killed with hydrogen cyanide before treatment with allethrin or the allethrin-synergist combination, the 24-hour penetration was essentially the same as that found for live flies tested in a similar manner.

Paper chromatographic analyses of the 24-hour rinses demonstrated that less than 4% of the total radiochemical impurities present in the C^{14}-allethrin had been absorbed by the flies. Of the available C^{14}-allethrin, more than 95% had penetrated the integument in 24 hours.

Internal Extracts.—When rinsed macerated flies were extracted with *n*-hexane and acidified acetone, only small amounts of radioactivity were recovered, ranging from 5 to 8% for allethrin alone and from 6 to 17% for allethrin plus piperonyl butoxide at the various time intervals. No attempt was made to determine the amount of radioactivity, if any, remaining in the residue. Total recoveries of radioactivity from all sources ranged from 61 to 92%, with the largest percentage recovered at the early intervals. Winteringham *et al.* (1955) recovered considerable radioactivity from the tissues of C^{14}-allethrin-treated flies by wet oxidation and collection of $C^{14}O_2$ that they were unable to extract with various organic solvents.

Excretion.—Twenty-four hours after treatment about 44% of the absorbed dose of C^{14}-allethrin was excreted in the feces (table 2). When allethrin and piperonyl butoxide were applied jointly, the rate of excretion was depressed as much as 30% at the early intervals and 18% at 24 hours.

Metabolism.—The metabolic fate of C^{14}-allethrin was investigated primarily by paper chromatographic analysis of the excreta collected at various intervals. When analyzed by the reverse-phase method, the bulk of excreted radioactivity ran as an elongated spot with an Rf value (0.59) intermediate between those of allethrin and its hydrolysis products. Cleaner, although not as quantitative, extracts obtained by Soxhlet extraction ran with an Rf (0.79) closer to that of chrysanthemum monocarboxylic acid (0.84). The two-stage normal phase method of Winteringham (1952) gave more indicative results. With this system 96 to 97% of excreted radioactivity remained near the point of application while only small quantities behaved as chrysanthemum monocarboxylic acid and allethrin. By the Shell Rubber Solvent-dimethylformamide system most of the radioactivity remained near the origin (Rf 0.06) of the chromatogram, with only trace amounts at the Rf of either the acid or allethrin. No qualitative differences were detected between the fecal extracts of allethrin and allethrin-synergist treated flies. The results of limited analyses of the 24-hour internal extracts were essentially the same as for the excreta, most of the radioactivity behaving as an unknown metabolite.

Discussion.—The penetration of allethrin through the integument of house flies was exceedingly rapid, 46% of the applied dose being absorbed in 4 hours. A near-quantitative absorption of the pure ester had occurred by 24 hours, but more than 96% of the C^{14}-labeled impurities was still on the exterior surface of the flies. The joint application of piperonyl butoxide and allethrin at a 1:1 ratio caused a slight decrease in the rate of allethrin penetration and an intial lag in knock-down time. This phenomenon has also been observed by earlier workers using piperonyl butoxide and C^{14} pyrethrins applied jointly to house flies (Lindquist *et al.* 1952, Earle 1952). Winteringham *et al.* (1955) found an even greater reduction in the absorption of pyrethroids by house flies when piperonyl cyclonene was applied jointly at ratios up to 10:1, and postulated that the difference was due to the dilution of the insecticide by the synergist on the insect integument.

Even more striking is the degradation and excretion of allethrin by house flies. Twenty-four hours after treatment about 44% of the absorbed dose was found in the excreta. Piperonyl butoxide depressed the rate of excretion as much as 30% which is probably correlated with the increased paralysis and mortality.

Paper chromatographic analysis of the fecal and homogenate extracts demonstrated only traces of unchanged allethrin and chrysanthemum monocarboxylic acid. The excreted radioactivity behaved almost quantitatively as a more polar compound(s) that remained near the point of application by the two normal-phase methods with approximately the same Rf as allethrolone. This would indicate the possibility of alterations of the intact molecule rather than hydrolysis of the ester linkage or, if hydrolysis occurs, the further degradation or conjugation of the free acids. Winteringham *et al.* (1955), using C^{14}-pyrethrins labeled on both the alcohol and acid portions of the molecule, were unable to detect any free acids in fly extracts. The metabolites from both pyrethrins and the alcohol-labeled allethrin consistently behaved as an alcohol.

Zeid *et al.* (1953) report finding chrysanthemum monocarboxylic acids and keto-alcohols plus unchanged esters and unknowns in various tissues removed from American cockroaches treated with randomly labeled C^{14}-pyrethrins. This would suggest the possibility of differing detoxication mechanisms for the pyrethroid insecticides in house flies and cockroaches.

Summary.—The fate of a pyrethroid insecticide in female DDT-resistant house flies (*Musca domestica* L.)

has been studied, using allethrin labeled with C^{14} on the chrysanthemum monocarboxylic acid moiety.

To study the absorption and excretion of allethrin or its degradation products quantitatively, a method was devised for attaching house flies in stationary positions throughout the experimental period.

Allethrin was rapidly absorbed, metabolized, and excreted. The synergist piperonyl butoxide applied jointly with allethrin at a 1:1 ratio retarded both absorption and excretion of the labeled insecticide. Almost all the absorbed dose of allethrin was metabolized by house flies in 24 hours. The bulk of the radioactivity present in the homogenates and excreta behaved as an unknown metabolite, only trace amounts of unchanged allethrin or free acids being detected. No qualitative differences in the metabolism of allethrin applied alone or jointly with piperonyl butoxide were found in these experiments.

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BOOK REVIEW

ANIMALS PARASITIC IN MAN. By Geoffrey Lapage. 302 pages, 75 black and white figures and plates. Penguin Books, Inc., 3300 Clipper Mill Road, Baltimore 11, Md. 95 cents.

This book is written primarily for medical students and doctors, especially for use in the Tropics, and for laymen interested in natural history, biology professors, and chemists. The object is to summarize the biology, morphology, and interrelationships of the important parasites affecting man, although frequent reference is made to parasitic forms in animals.

The first two chapters define the different types of parasitism and briefly discuss the various Phyla of the animal kingdom in which parasitic forms are found. General life histories of these forms are presented.

Five chapters are devoted to details of host-parasite relationships and the behavior of such parasites as the protoza, flukes, filariae, nematodes, and tapeworms. The author uses a unique classification system, referring to parasites having a direct life cycle as "lone hands" and those requiring one or more intermediate hosts as "animals twice or three times parasitic in one life." These chapters have excellent illustrations.

The chapter dealing with malarial parasites is brief but well presented. The author describes the disease in man and the huge financial loss due to this age-old malady, but devotes only about a page to the use of insecticides for control of *Anopheles* vectors, one of the most interesting research and operational accomplishments in man's fight against this debilitating disease. Notwithstanding the brevity of this discussion, it is up to date, even including authoritative statements on insect resistance to insecticides.

The human trypanosomes are discussed thoroughly, and the insect vectors are indicated.

Insects that affect man directly as well as being disease vectors are discussed in the chapter headed "The Skin as a Home or a Source of Food." The morphologies of the various forms together with brief life histories are very well presented. Insects, ticks, mites, fleas, lice, bloodsucking bugs, the nonbiting house flies, blow flies, and warbles are dealt with in this chapter. The author points out that the human body louse is an important vector of some human diseases, including endemic typhus fever. It would have been appropriate to mention here the excellent control methods developed for the protection of military and civilian personnel against this louse during World War II. These methods represent an illustrious accomplishment in the control of insect vectors of serious diseases by the use of insecticides.

A reviewer usually finds minor errors or imperfections, and attention is called to the statement that cattle warbles feed on pus in the cysts just under the skin. According to the reviewer's knowledge, no one has proved just what these larvae feed on for they live in an environment practically free of pathogenic bacteria. The larvae produce antibacterial activity in the cysts.

This authoritative and well illustrated book will serve as a very useful reference for those interested in the broad complex of parasites affecting man.

A. W. LINDQUIST

SCIENTIFIC NOTES

A Laboratory Technique for Obtaining Quantities of Comparable Hessian-Fly Infested and Uninfested Wheat Plants[1]

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The present study was one phase of a broad investigation of biochemical factors involved in the resistance of wheat plants to the hessian fly (*Phytophaga destructor* (Say)). The object was to provide quantities of comparable hessian-fly infested and uninfested wheat plants for biochemical analyses.

Soil in which wheat plants are grown in the greenhouse may be assumed to be comparable providing it is blended thoroughly before planting, However, blending the soil properly is a difficult task. Furthermore, the amount of plant material that can be grown per unit area of soil is small and there is always danger from build-up of disease organisms in the soil. Uniform watering also is not always obtained. Although no growth medium or set of conditions will guarantee identical qualitative or quantitative characteristics of the plants, the hydroponic technique provides the most satisfactory nutrient and water source. With this technique the maximum amount of plant material can be produced per unit area. The method has been generally successful and much better than the use of soil.

MATERIALS AND METHODS.—The hydroponic tank assembly (fig. 1) consisted of a germination tray, a hydroponic tank, and a seed frame. The germination tray measured 1×14×30 inches. The seed frame measured 1×15×31 inches and was designed to fit on the germination tray and hydroponic tank without danger of displacement. This was made possible by angular metal pieces riveted to each end and pointed downward to rest inside the germination tray of the hydroponic tank. The entire assembly was constructed from 22-gauge galvanized iron except for the bottom of the seed frame which was constructed of 0.125-inch mesh galvanized hardware cloth. All joints were riveted and soldered. A hardware-cloth covered wooden seed frame also was satisfactory.

The exposed surfaces were painted with two coats of asphaltum varnish[2,3] to prevent interaction between the zinc-coated iron and the nutrient solution. Two other types of coatings including a plastic resin paint manufactured under the name of Jet-Kote x-3m[4] and a polyethylene plastic sheet[5] were used.

A 0.625-inch soft copper drain tube and a 0.25-inch soft copper aeration tube having 0.033-inch orifices at 8-inch intervals were installed in the hydroponic tank. The copper pieces were not painted.

Aeration was provided by supplying air continuously at 20 lbs. pressure to the nutrient solution. A manifold, shown by an arrow in figure 2, consisted of a 1-inch electrical conduit in which holes were drilled and ¼-inch copper tubes soldered at suitable intervals. By means of rubber hose connecting these copper tubes with the aeration tubes, air delivered to the manifold was distributed uniformly to the various tanks.

Prior to planting, the wheat seeds were dusted with Spergon,[6] to prevent decay due to fungi. Approximately 300 dusted seeds were distributed in 6 rows 1.5 inches apart on the hardware cloth of the seed frame. The seeds were covered with 0.5 inch of coarse vermiculite to retain moisture and exclude light. The seed frame was then placed on top of the germination tray, which had been filled with vermiculite and as much tap water added as possible. After 15 minutes the excess water was poured off. The vermiculite covering the seeds was sprinkled with water whenever it became dry.

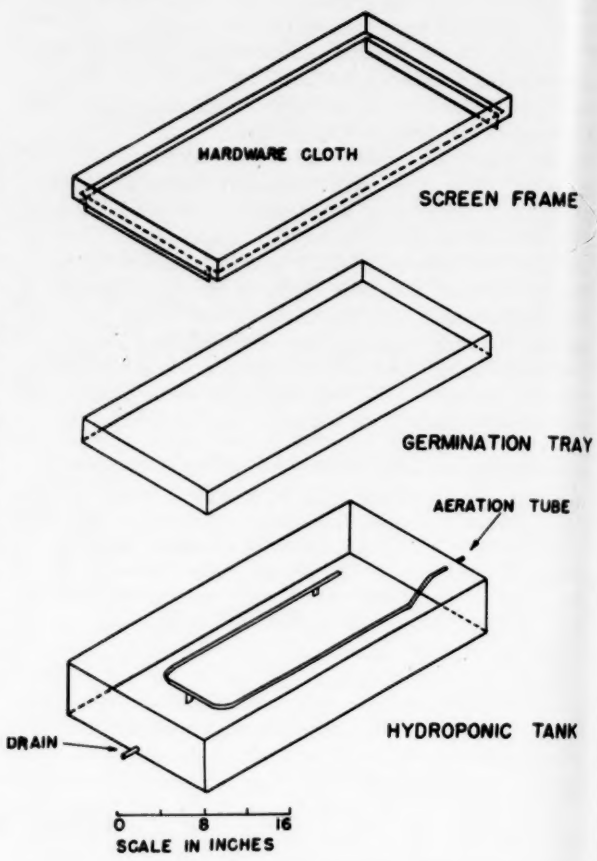

FIG. 1.—Hydroponic tank assembly.

When the wheat plants were 10 days old, or approximately 3 inches tall, the seed frame was removed from the germination tray. Excess vermiculite was removed from the roots and the seed frame containing the plants was set on top of the hydroponic tank. The tank was filled to within 1 inch of the top with 7.5 gallons of tap water to which 15 grams of Hyponex[7] per gallon was added as the source of plant nutrients. The hydrogen-ion concentration of the nutrient solution was adjusted to pH 5.0 to 6.0 with sodium hydroxide solution each time the nutrient solution was changed. Ferric tartrate at a concentration of 5 p.p.m. of iron (one ml. of a 0.5% solution per quart of nutrient solution) was added to the nutrient solution every 3 days to prevent yellowing of the plants due to insufficient iron. Verenol iron chelate[8] also was used in later experiments as a source of iron. It is readily soluble in cold water and does not require neutralization. Versenol iron chelate was used at a concentration of 5 p.p.m. of iron, obtained by mixing 211 mg. of the product per gallon of

[1] Cooperative investigations between the Crops Research Division and the Entomology Research Division, Agr. Res. Service, U.S.D.A., and the Department of Flour & Feed Milling Industries, Kansas Agricultural Experiment Station, Manhattan. Contribution No. 280, Department of Flour & Milling Industries, Kansas Agricultural Experiment Station, Manhattan. This work was supported in part by a grant from the National Science Foundation. Accepted for publication April 9, 1957.

[2] Pratt and Lambert, Inc., New York, N. Y.

[3] The mention of any firm name or commercial product by trade name does not constitute endorsement by the U. S. Department of Agriculture.

[4] Furane Plastics, Los Angeles 39, Calif.

[5] Custom Products, Inc., Chicago, Ill.

[6] U. S. Rubber Co., New York, N. Y.

[7] Hydroponic Chemical Co., Inc., Copley, Ohio.

[8] Dow Chemical Co., Midland, Mich.

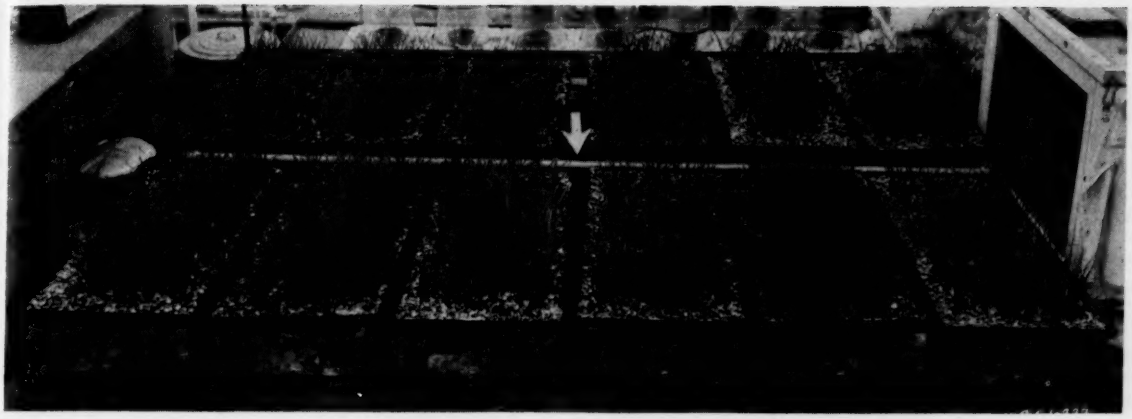

FIG. 2.—Wheat plants growing in hydroponic solution.

water. It was added only when the nutrient solution was changed.

Twelve of the tanks containing the 3-week-old plants were divided into 2 lots of 6 tanks each. Each lot was covered with a cheesecloth tent tightly fitted to prevent hessian flies from either entering or leaving the enclosure. In one lot, 10,000 adult flies, obtained from stock cultures supplied by the Entomology Research Division's hessian-fly laboratory at Manhattan, Kansas, were released inside the tent over the plants. The other lot was kept free of infestation. The use of the two tents insured the same degree of shading for the infested and uninfested wheat plants. The cheesecloth tents were kept damp to help maintain a relative humidity of 75 to 85% (McColloch 1923) inside the enclosures. The tents were removed when all danger of infestation of the control plants had passed.

The water level was maintained at 7.5 gallons for the first 2 weeks by the daily addition of tap water. Once a week the leaves of the plants were sprinkled with water.[9] At the end of the first 2 weeks the old nutrient solution was drained and fresh solution added. The volume of water was reduced to 5.5 gallons with a corresponding reduction in the quantity of nutrient. The lowering of the nutrient solution level in the culture tanks was necessary to insure a sufficiently high oxygen content around the roots of the plants. Thereafter, the nutrient solution was exchanged for fresh solution every 2 weeks and the volume maintained at 5.5 gallons. The plants were harvested after growing for 6 weeks. At this time they showed all the symptoms of infestation with hessian fly.

RESULTS AND DISCUSSION.—The wheat plants grown using Hyponex yellowed about the middle of the second week. This yellowing was corrected by the addition of ferric tartrate solution every 3 days. In preparing the ferric tartrate solution, it was

[9] As instructed in the Hyponex plant food brochure. Hydroponic Chemical Co., Inc., Copley, Ohio.

FIG. 3.—Wheat plants growing in hydroponic tanks which have been protected by different materials: A, painted with asphaltum-varnish; B, covered with a new sheet of polyethylene plastic; C, covered with a sheet of polyethylene plastic which had been thoroughly soaked and washed prior to use.

necessary to heat and adjust the pH of the solution to 9.0 with sodium hydroxide to insure complete solubility. The best source of iron supplement used was Versenol iron chelate.

At the end of the first 6 weeks growing period the asphaltum-varnished hydroponic tanks had chipped and peeled and required repainting. The plastic resin paint was durable and quite satisfactory. Before any paint is applied care should be taken to clean thoroughly the surface to be covered. The new polyethylene plastic sheeting material contained a toxic substance which killed the plants as seen in tank B of figure 3. Plastic which had been used once or had been thoroughly soaked prior to use was satisfactory as seen by the healthly plants in tank C. These plants were comparable with the plants grown in tank A which had been painted with asphaltum varnish. Since the use of plastic sheets interfered with the use of the aeration and drain tubes, the tank assembly should be redesigned if the use of plastic sheets is contemplated.

Satisfactory growth and plant characteristics were obtained at early stages of development with Rival and Pawnee wheat when infested with Western and Missouri hessian-fly biotypes, respectively. Both uninfested and infested plants grown in Hyponex were normal and compared favorably in physical characteristics with plants grown in sand or soil cultures. Other sources of hydroponic chemicals might be equally satisfactory. There was 95% infestation of the Rival wheat and 94% infestation of the Pawnee wheat plants under the infested cages. The eggs, larvae, and flaxseeds appeared to be normal.

This technique for growing plants has been applied successfully to plant selections being tested for resistance to hessian fly. Plant irregularities due to other insects or to diseases that occur in soil cultures can be avoided while maintaining a critical control of plant and insect development. By using a cardboard template, 50 different varieties can be grouped in each tank. Thus, using 12 tanks, 600 varieties can be tested at any one time in a space measuring 6×8 feet.

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Relation of Particle Size to the Penetration of Subterranean Termites through Barriers of Sand or Cinders[1]

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Western subterranean termites, *Reticulitermes hesperus* Banks, can be maintained for a month or more in moist soil or very fine sand in test tubes. In the present experiments, a sand that passed through a 50-mesh screen was used. The sand was poured into a 1×8 inch glass test tube in the bottom of which a narrow slit was cut by means of a glass-cutting wheel. At the bottom of the glass tube was placed a copper or plastic screen sufficiently fine to prevent the dry sand from sifting through and to prevent termites from leaving the tube through the slit. The sand was poured to within 2 inches of the top of the tube. It was moistened by placing the bottom of the tube in water and allowing the water to rise by capillarity to half the height of the column of sand. The tube was then removed and water already within the sand eventually rose to the top of the column.

Capillarity provides a convenient means of moistening sand or other media to approximately the optimum condition for subterranean termites, for the quantity of water is automatically adjusted to the variations in particle size (or pore size) in the medium selected. Another advantage is that a moisture gradient is established. The initial gradient is to some extent maintained by evaporation of moisture from the top of the column. This allows the termites to find their optimum moisture requirements periodically if portions of the medium are less than optimum in this respect.

Pence (1957) has demonstrated that, if a piece of wood (for food) is attached to a porous column of sufficient height so that a continuous moisture gradient, based on capillarity alone, can be established, a termite colony can be maintained indefinitely in such an environment. However, in the sand columns as used in the present experiments, the termites can be maintained without appreciable mortality, and in a condition of continuous activity, for over a month. If an applicator stick is thrust into the sand next to the glass, the termites are provided with food and can be kept alive for even longer periods.

After the moisture had reached the top of a sand column, a tunnel was made in the sand next to the glass by means of a hardwood applicator. The termites entered this tunnel and bored through the sand in all directions, remaining next to the glass surface, where they could be easily observed (fig. 1, A).

If an additional inch of sand of the same particle size is poured on top of the original column and moistened, the termites will riddle it with tunnels within a few hours, for they have a strong urge to burrow upwards. This habit provides an excellent means of testing insecticide-treated soils when they are placed above the termite-infested sand, for termites will readily enter a medium of smaller particle sizes than the one they are infesting. Soils treated with the insecticides and at the dosages usually employed in soil treatment were never penetrated in tests made in this laboratory. However, treated soils will form the subject of a later paper.

Termites are reluctant to burrow from a sand of one range of particle sizes to a sand of larger particles as, for example, from sand of 50 to 100 mesh to sand of 32 to 50 mesh. However, they will eventually tunnel their way through the coarser sand. They can be induced to penetrate sands of increasingly greater mesh size up to approximately 16-mesh.

METHOD OF TUNNELING.—Subterranean termites cannot tunnel through dry sand of small particle size because their tunnels collapse. They can proceed through dry sand of large particle size (above 3 mm. in diameter) by passing through the interstices.

In moist sand subterranean termites progress by pushing heads forward through the sand, then pressing the sand particles to either side, primarily with their heads and the exterior surfaces of the mandibles, although the entire body appears to be used to some extent for this purpose. The smaller sand particles are taken into the buccal cavity, then placed along the walls of the tunnel to make a smooth and tightly-sealed surface. The sand particles are mixed with a gluey substance in the mouth of the termite, then pushed out by means of the large hypopharynx. This organ is greatly broadened anteriorly, evidently as an adaptation for pushing out sand or earth particles and spreading them over a relatively broad surface. When they carry sand to the surface in order to build shelter tubes, the termites transport the smaller particles in their buccal cavities and grasp the larger ones with their mandibles. Although the span of the mandibles is only about ½ millimeter, by grasping the edge of a sand particle, termites can move particles of about a millimeter in length.

AN EFFECTIVE SAND BARRIER.—Sand with particles ranging in size so that not more than 5% will be retained on a 10-mesh screen and not more than 5% will pass through a 16-mesh screen forms an impenetrable barrier to subterranean termites. The smaller particles are too large to be pushed aside or carried and the larger particles are not so large as to leave spaces through which the termites can make their way. However, there is a much greater latitude in the range of effective particle sizes, particularly in the direction of the larger sizes, if the sand is tamped so as to cause the spaces between the larger particles to be decreased to a minimum of volume. For example, a sand is prepared commercially which is referred to as "C 6 to 16," the numbers indi-

[1] Accepted for publication May 21, 1957.

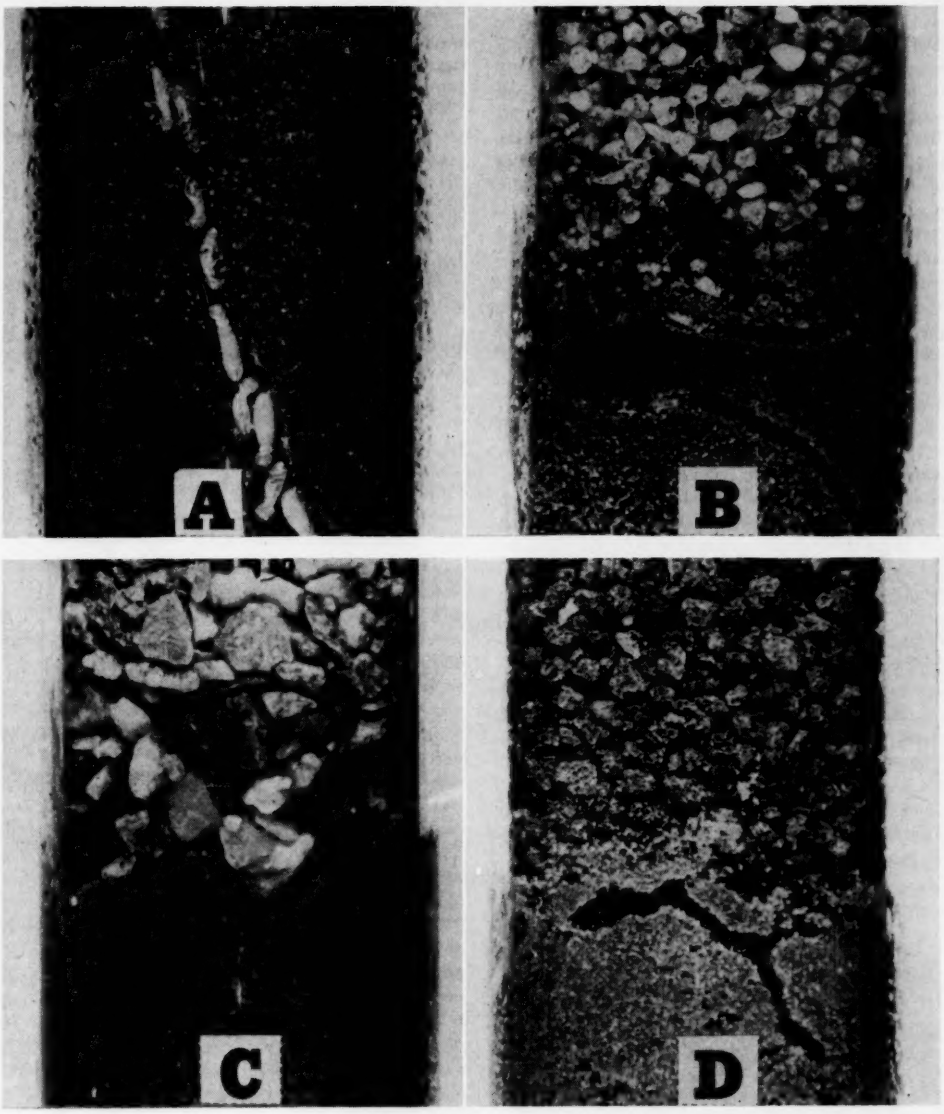

FIG. 1.—Subterranean termite tunnels in columns of sand or cinders in glass tubes. A, typical vertical tunnel in 50- to 100-mesh sand; B, C and D, vertical progress of tunnels stopped by 10- to 16-mesh sand, 6- to 16-mesh sand (packed), and 10- to 16-mesh crushed cinders, respectively.

cating the range of mesh size. Although the larger particles in this sand are above the optimum size if the sand is loosely packed, this sand becomes a highly effective barrier if it is tamped sufficiently to decrease the interstices to a minimum. It has been used in this manner in cooperative experiments with termite operators for sealing off earth-filled porches, patios, steps, etc. from the main foundations of houses. It is presumed that the sand will not part away from the foundation as concrete does from stress of subsidence of earth, earthquakes, sonic booms, and other factors.

Figure 1, A, shows a vertical tunnel in a column of 50- to 100-mesh sand that was burrowed out by termites at the rate of about 1 inch per hour. Figure 1, B, shows how the same termites burrowed back and forth horizontally at the interface of the 50- to 100-mesh sand and a sand ranging in particle size from 10- to to 16-mesh, untamped. No progress was made into the coarse sand during the 3 weeks that the termites were observed, although they never ceased their efforts to move upward. Other tests showed that termites were likewise unable to move sideways or downward through this grade of sand.

Figure 1, C, shows a sand of particle sizes ranging from 6- to 16-mesh, but tamped to decrease intersticial space. This sanp proved to be effective as a barrier when it was sufficiently tamped.

Crushed volcanic cinders, which is about 30% lighter than ordinary sand, is also effective as a barrier to termites. Figure 1, D, shows a 1-inch barrier of 10- to 16-mesh ground cinders placed on termite-infested sand. As in B and C, the vertical progress of the termites was terminated abruptly when they reached the larger particles. The lateral tunnels at that point represent their futile effort to penetrate the barrier at various points.

Finely divided slag, known as "sinter," a by-product in steel production, forms an effective termite barrier if the particles of the required range of sizes are screened out.

Crushed cinders of particle sizes ranging from 10- to 18-mesh are being provided for field experiments. In these experiments, ground on which conventional or slab-on-ground houses are to be built is covered with cinders in critical areas, such as around the inside of the foundation, around utility pipes, and where

earth-filled patios, terraces, porches, steps, and stoops are to be constructed. The "C 6 to 16" sand, properly tamped, is also being used in this connection. By working in tracts in which the preconstruction conditions of the soil and the type of structure are approximately uniform for treated and untreated houses, significant data on the feasibility and effectiveness of volcanic cinders or sand as a barrier to termites under practical conditions should be obtainable.

TESTS OF VAPOR- AND TERMITE BARRIERS OF ASPHALT.—Layers of asphalt from emulsions or "cut back" solutions of asphalt, such as are used as vapor barriers in slab-on-ground construction, were placed on termite-infested sand. After the asphalt had solidified, the test tubes were closed with rubber stoppers and the relative effectiveness of the different types of asphalt in preventing the passage of vapor was ascertained. Moisture condensed on the inner walls of the glass tubes above the layer of asphalt when vapor passed through.

The termites immediately attempted to bite their way through the asphalt layers, but since they did not concentrate their attack at any single point, it required 3 or 4 weeks for them to make their way through a ¼ inch layer of asphalt. When 1% of such chlorinated hydrocarbons as chlordane, DDT, dieldrin, lindane, methoxychlor, or toxaphene was incorporated into the asphalt, the termites did not attempt to penetrate the layer. Consequently, insecticide-treated asphalt as a combination vapor- and termite barrier is being tested along with the cinders and sand, in some of the field experiments described above. In these experiments, the insecticide-treated asphalt emulsions are applied to areas inside the foundation and a 1-inch layer of cinders or sand is placed on the ground in earth-filled extensions of the foundation, such as patios and porches.

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Effect of Mouse-Control Spray of Endrin on Insect Life in Orchard Ground Cover[1]

W. S. HOUGH, *Virginia Agricultural Experiment Station, Winchester*

Ground spraying with endrin to control orchard mice has become an established practice within recent years in many orchards of Virginia (Horsfall 1956). The current recommendation is that spraying be done between mid-October and mid-March and that an emulsifiable endrin (1.6 lb. of endrin per gallon) be diluted at the rate of 14 pints in 500 gallons of spray mixture.

The question as to the effect of the endrin ground-spray on insects hibernating in or among the fallen apple leaves arose in connection with a study of the natural enemies of the unspotted leaf miner, *Callisto geminatella* (Packard). Most of the natural enemies of the leaf miner and many of the parasites of another important apple pest, the red-banded leaf roller (*Argyrotaenia velutinana* (Wlk.)), overwinter in or among the fallen apple leaves. Overwintering stages of the leaf miner (larva and pupa) and the leaf roller (pupa) are found among the fallen apple leaves. Consequently, information concerning the effect of the mouse-control spray on insect life hibernating in the orchard ground cover is of practical importance to orchardists.

In March fallen apple leaves were collected and brought to the insectary from an orchard infested by the leaf miner and the red-banded leaf roller. Some of the leaves were placed in cloth-lined cages to observe emergence of insects from unsprayed leaves. The remainder of the leaves were spread on trays made of hardware cloth and thoroughly sprayed with the endrin spray mixture at the dilution recommended for mouse control. Three days later the sprayed leaves were placed in cloth-lined cages to observe subsequent emergence of insects. On March 16 a second series of tests was inaugurated by spraying some of the ground cover in the orchard. Two days later leaves from the treated and untreated areas were collected and placed in cloth-lined cages for observance of emergence of insects. Most of the cages were kept in the laboratory at room temperature to speed insect emergence. Except for the time of emergence, no difference was noted between emergence of insects from the material kept either at outdoor temperatures or at room temperatures.

Table 1.—Emergence of insects from apple leaves of an orchard ground cover which received the endrin-spray treatment for mouse control, as compared with emergence from unsprayed leaves. Winchester, Va., 1957.

	TREATMENT OF GROUND COVER	
	Endrin 14 Pt. in 500 Gal.	Unsprayed
Lepidoptera		
Red-banded leaf roller	6	0
Unspotted leaf miner	8	1
Hymenoptera		
Parasites	254	269
Ants	8	2
Bee (Andrenidae)	0	1
Diptera (Syrphidae)	5	3
Coleoptera (Dermestidae)	1	0

A summary of the record of emergence is given in table 1. Nearly all of the parasitic Hymenoptera were members of genera having species known to be parasitic on the unspotted leaf miner or the red-banded leaf roller or occurred as hyperparasites in the parasite complex associated with one or both of these insects. The principal groups listed in order of abundance were braconids of the genus *Apanteles*, ichneumonids of several genera, but mostly *Gelis*, eulophids including *Sympiesis*, *Elachertus*, and probably *Horismenus*, and some specimens believed to be members of the family Pteromalidae. More than 32% (169 specimens) of the parasitic Hymenoptera taken from the cages were *Apanteles*. The most important parasite of the unspotted leaf miner observed during the past 3 years was *Apanteles ornigis* Weed. This species accounted for most of the *Apanteles* recorded from both the treated and untreated leaves.

Inasmuch as the volume of endrin-sprayed leaves was about three times greater than the volume of unsprayed leaves, it was assumed that the number of insects emerging from the sprayed leaves should exceed the number emerging from the unsprayed leaves, if the endrin treatment did not destroy or reduce the insect population. Except for the ichneumonids, this was the case where several insects of a kind were recorded. For example, of the 102 specimens of ichneumonids collected from the cages, 49% were from endrin-sprayed leaves and 51% from unsprayed leaves; but of the 169 specimens of *Apanteles* collected, 57% were from endrin-sprayed leaves and 43% from unsprayed leaves. The fact that the proportion of ichneumonids from the sprayed leaves was slightly less than from the unsprayed leaves may indicate that endrin reduced slightly the ichneumonid population.

CONCLUSION.—No evidence was obtained to indicate that the endrin-spray treatment of the orchard ground cover adversely affected the over-wintering stages of the unspotted leaf miner and the red-banded leaf roller or any of their Hymenopterous parasites or hyperparasites found in the ground cover, except that adults of the family Ichneumonidae may be slightly re-

[1] Accepted for publication June 5, 1957.

duced in numbers. Flower flies (Syrphidae) were not adversely affected according to the limited data available.

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A Rapid Method for Counting Larvae of the Confused Flour Beetle[1]

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In studies involving the confused flour beetle (*Tribolium confusum* Duv.) it is often necessary to be able to determine the number of larvae in various test media such as corn meal or flour. This can be a time-consuming operation when done by the simple technique of examining representative fields with a dissecting microscope. A method that has been used for separating the larvae of various beetles from rearing media is the simple one of rolling the media on rough paper (Fletcher 1942). By this method the larvae will tend to cling to the paper when it is tilted to roll off the inert material. Laudani (1948) pointed out that by using this method many larvae are swept off when the paper is tilted, necessitating the repetition of the operation many times before most of the larvae are separated from the medium. We have developed a modification of this method which appears to be both rapid and accurate. In order to test the accuracy of the method the following experiments were undertaken.

A medium consisting of dry, white corn meal and cracked grain was prepared. To this was added an uncounted number of *T. confusum* adults. After a period of 7 to 10 days, the adults were driven off by the application of a strong light source to the scattered medium. The adult-free medium was then thoroughly mixed for sampling. Five each of 1-, 2-, and 5-gram samples, and three 10-gram samples were then weighed out. Each individual sample was sprinkled on a $5\frac{1}{2}'' \times 9''$ piece of black construction paper ($11'' \times 9''$ in the case of the 10-gram samples) and spread out by gentle shaking of the paper. After a period of approximately 40 seconds, the material was shaken gently onto another piece of construction paper. Most of the larvae remained clinging to the first sheet of paper. The number of larvae was recorded and the larvae removed. This procedure was repeated twice and after the third transfer the medium was transferred to a glass container of such size that the material covered the bottom to a depth of approximately $\frac{1}{2}$ inch. After a period of 21 days at room temperature the number of adults appearing in each sample was recorded.

The number of larvae recovered in the three transfers was added to the number of adults obtained after incubation for 21 days to obtain the total number of larvae present in the original sample. From this value, the per cent recovery after each transfer was calculated. The results are given in table 1. For the 1-, 2- and 5-gram samples, most of the larvae were separated in the first two transfers, whereas for the 10-gram sample four separations were required. With this technique as described not more than 5% were missed, if one includes the extra separations required for the 10-gram sample. Considering the simplicity of the procedure and its apparent accuracy, it would seem that for small samples at least, the method could be recommended. It should be pointed out, however, that only live larvae are separated by the technique. We used the method for evaluating the toxic effect of various chemicals on *T. confusum* adults and larvae; thus, only live larvae needed to be counted.

Table 1.—Recovery of larvae of *Tribolium confusum* from corn meal.

WEIGHT OF SAMPLE (GM.)	TOTAL LARVAE	PER CENT RECOVERY IN TRANSFER INDICATED			
		1st	2nd	3rd	4th
1	23.2[a]	81.0	12.9	2.6	—
2	36.8[a]	79.4	17.4	0.5	—
5	88.4[a]	77.4	14.3	5.0	—
10	172.7[b]	68.5	10.6	8.9	7.4

[a] Average of five samples.
[b] Average of three samples.

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[1] Accepted for publication June 7, 1957.

Flies Attracted to the Giant Star Flower (*Stapelia gigantea* N. E. Br.)[1]

W. E. SNOW[2]

During the latter part of September 1956, Dr. T. F. Hall of Sheffield, Alabama, noted an unusual number of blowflies resting on the porch screens of his residence. An ornamental plant called the giant star flower or carrion flower (*Stapelia gigantea* N. E. Br., Family Aesclepiadaceae) was then in bloom on the porch. When the plant was removed to the backyard, Dr. Hall noted that flies tended to congregate around the flowers and that some were, in fact, depositing eggs in the base of the corolla.

The disc-like flower is starshaped with a shallow depression in the center. Bailey (1953) describes the inner surface as transversely rugulose, pale-purple-hairy, light ochre-yellow, everywhere marked with transverse crimson lines. The corolla measures 11 to 16 inches in diameter with individual lobes 4 to $6\frac{1}{2}$ inches in length. The plant is native to South Africa where it is also known to attract flies. In a recent discussion with Dr. W. H. R. Lumsden, Entebbe, Uganda, he stated that he knew of no published lists on the species of flies visiting *S. gigantea*. It therefore seemed worthwhile to record those species found associated with the flower in the U. S. A.

On the afternoon of October 4, 1956, the writer collected the following insects from two flowers of the plant in the yard of the Hall's residence between 1:30 and 2:30 p.m. with temperature in the 70's and sunlit conditions:

Diptera	
Calliphoridae	
Phaenicia pallescens (Shannon)	7
Phormia regina (Meig.)	11
Muscidae	
Fannia sp.	2
Ophyra leucostoma (Wied.)	1
Sarcophagidae	
Sarcophaga spp.	2
Chloropidae	
Hippelates pallipes (Lw.)	18
Hippelates pusio Lw.	5
Hymenoptera	
Chalcididae	
Brachymeria fonscolombei (Duf.) (a parasite of blowflies and other muscoids)	1

In addition to the insects captured by aspirator, 40 eggs and 2 muscoid larvae were counted among hairs on the upper sur-

[1] Accepted for publication June 11, 1957.
[2] Biology Section, Malaria Control Branch, Division of Health and Safety, Tennessee Valley Authority, Wilson Dam, Alabama.

face of the lobes and from among stamens in the base of the corolla. The calliphorid flies were responsible for some of the eggs, but it was not determined whether the larvae present were deposited by the sarcophagids or had emerged from eggs previously deposited. The flower bearing the eggs and larvae was returned to the laboratory, but no further development was noted on the leaf. Apparently odor from the flower stimulates oviposition, but the medium is not suitable for development of the immature stages. Considering the types of flies reported in this observation, the attractant in the flower of *S. gigantea* might be of use in fly control.

Thanks are due the following specialists of the Insect Identification and Parasite Introduction Section, Agricultural Research Service, U. S. Department of Agriculture, for determination of the respective groups: C. W. Sabrosky, Chloropidae; R. H. Foote, Muscidae, in part; and B. D. Burks, Chalcididae.

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Honey Bee[1] Colony Weight Changes in Relation to Malathion Bait Spray Applications[2]

D. O. WOLFENBARGER and FRANK A. ROBINSON, *Florida Agricultural Experiment Stations*

In the campaign to eradicate the Mediterranean fruit fly, *Ceratitis capitata* (Wied.), concentrated spray mixtures of malathion were applied at the rate of ½ pound actual toxicant per acre at 10-day intervals. These spray applications were made with single- and multi-engine airplanes to many square-mile sections and even entire counties so the Mediterranean fruit flies would be attracted to and feed on the poison.

Protests were made against the use of such wide-spread aerial applications because it was often stated that much wild life, domestic animals and perhaps even man would be destroyed. Although attempts were made to allay such fears, the exact nature and extent of effects of the spray applications were not known.

Owing to the importance of the honey bee to agriculture, consideration must be given to the effects of insecticidal chemicals applied for the control of harmful insects. Knowlton (1954) suggested proper timing of spray applications used in insect control practices to protect honey bees. Weaver (1949) found that BHC dust mixtures were considerably more toxic than DDT, chlordane or toxaphene, thus illustrating differences in toxicity of different insecticides to honey bees. An inquiry, made by Eckert (1954), "How will new insecticide affect bees?", refers to the phosphatic insecticide, demeton, but may be asked of all insecticides. Toxicity of several new insecticides was studied by Anderson & Tuft (1952) where it was shown that malathion, parathion, BHC, EPN, and lindane were more toxic to bees than chlordane, heptachlor, dieldrin, aldrin, DDT, methoxychlor, Perthane, and TDE. They showed that 1% malathion dust applied to bees gave 100% mortality within 20 minutes, and that bees confined in cages with flower bouquets dusted with 1% malathion dust were all killed within 5 hours. Malathion and parathion as 1% dust mixtures were about equal in toxicity to bees where it was applied to the insects in cages and to flower bouquets on which bees were caged. Malathion and parathion were regarded as two of the most toxic of insecticides to honey bees. Nevertheless, Robinson & Kelsheimer (1957) found that colony weights increased during and after malathion-bait spray application for Mediterranean fruit fly eradication.

PROCEDURE.—Daily colony weights of one to two colonies of bees were recorded over the last 5 years. These daily weight records and the data on airplane spray dates were used for studying effects of the poison bait mixtures applied for Mediterranean fruit fly eradication.

Effects of spraying the colony locations and surrounding areas might be expected to be measured by (1) loss of bees, as observed by dead insects at the colony entrances, (2) abnormal loss of weight of the colonies or (3) some benefits might be observed or (4) there might be no change. Bees might fall at distances from the colony and never be observed. Such losses, if severe, should give responses in reduced weights of the colony. Losses in weight could be attributed to (1) reduced numbers of bees returning to the colonies, (2) reduced harvest of nectar, pollen, water and propolis, or, both (1) and (2). If the sprays were not harmful or had insignificant effects on the colonies, no change in weights would be observed over periods of a week or more.

RESULTS.—Dead bees about the colony entrances, always present in small numbers, were never greater in number after the spray applications than on previous days. Data are given for specific periods, based on the days spray applications were made during the summer of 1956. Honey bee colony gains or losses, in pounds per day, are given for the specific periods each year. Average colony weight changes in the months of June, July and August are given in tables 1 and 2 for the same periods in 1952, 1953, 1954, and 1955 as for 1956. Colony weight changes in 1956, the year of wide-spread malathion-bait applications, may be compared with weight changes of the 4 previous years.

Losses in weight during the months of June, July and August were usually observed over the 5 years of observations (table 1) and are attributed in part to the rainy season. The greatest average daily loss (0.54 lb.) was in 1955, the lowest (0.13 lb.) was in 1956. The average loss for the 4-year period was 0.34 lb., compared with 0.13 lb. found in 1956.

[1] *Apis mellifera* L.

[2] Florida Agricultural Experiment Stations Journal Series, No. 617. Accepted for publication June 17, 1957.

Table 1.—Average colony weight changes per day by 10-day periods[a] 1952–1955, without spraying and after spraying in 1956.

YEAR	TEN-DAY PERIODS BEGINNING							
	June 18	June 28	July 9	July 19	July 30	Aug. 9	Aug. 20	AVERAGE
1952	−0.45	−0.25	−0.25	−0.15	−0.20	+0.10	−0.15	−0.19
1953	−0.40	−0.45	−0.50	−0.40	−0.15	−0.20	−0.20	−0.33
1954	−0.80	−0.45	−0.45	−0.15	−0.05	−0.25	−0.05	−0.31
1955[b]	−0.80	−0.48	+0.10	−1.05	−0.55	−0.48	−0.50	−0.54
Average	−0.61	−0.41	−0.28	−0.44	−0.24	−0.21	−0.23	−0.34
1956[b]	−0.10	−0.33	−0.08	−0.15	−0.08	−0.02	−0.12	−0.13

[a] In some cases more than 10 days elasped between spray dates.
[b] Two colonies were weighed each day; 1952–54 one colony was weighed each day.

Table 2.—Average colony weight changes on 10 consecutive days, 1952–1955 inclusive, without spraying, and after spraying in 1956.

Days After Spray Application	Year Records Were Taken					
	1952	1953	1954	1955	Average	1956
1st	−0.14	−0.43	−0.57	−0.11	−0.26	−0.39
2nd	−0.21	−0.29	−0.21	−1.00	−0.43	+0.18
3rd	−0.14	−0.29	−0.36	−0.82	−0.40	−0.54
4th	0	−0.50	−0.21	−0.86	−0.39	+0.54
5th	−0.29	−0.14	−0.14	+0.32	−0.06	−0.86
6th	−0.36	−0.57	−0.43	+0.18	−0.30	+0.21
7th	−0.21	−0.07	−0.29	−0.68	−0.31	−0.36
8th	−0.36	−0.57	−0.50	−1.68	−0.78	−0.29
9th	−0.14	−0.36	−0.21	−1.96	−0.67	+0.11
10th	−0.07	−0.07	−0.21	+1.04	+01.7	+0.14
Averages	−0.19	−0.33	−0.31	−0.54	−0.36	−0.13

Colony weight changes from 1952 through 1955 for the same periods following spray applications in 1956 are given in table 2. These data show losses more frequently than gains over the 10-day periods in the years 1952 to 1955. Gains are more frequent, however, than losses in 1956. There is, moreover, no relationship between colony weight changes and spray date.

SUMMARY.—Malathion-bait spray applications made for Mediterranean fruit fly eradication were found to have no relationship to honey bee colony weight changes. In view of the recognized toxicity of malathion to the honey bee, it is apparent that insufficient malathion was applied to the colony site and to the adjacent areas to have resulted in colony weight reductions.

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Control of the Myocoptic Mange Mite, *Myocoptes musculinus* (Koch), on Laboratory Mice[1]

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Recently attention was called to a mite infestation involving the white mouse colony in the Third U. S. Army Medical Laboratory's animal house. Several mice were sacrificed, washed and the residue examined to determine the extent of the infestation. Population totals in excess of 500 mites were observed on all mice sacrificed. Determination was made as *Myocoptes musculinus* (Koch), belonging to the Listrophoridae.[3]

The mice are maintained in glass animal jars of approximately 2-gallon size covered with 0.25-inch hardware cloth lids. The litter is peat moss to a depth of approximately 3 inches. Food is scattered on the litter and water is made available by means of a glass tube leading from an inverted half-pint bottle set upon the jar lid.

Preliminary tests were initiated using 2% malathion as a dip and as a litter treatment. The malathion produced no visible adverse symptoms in the mice and both methods of applying it proved equally effective in these trials.

The actual control of the infestation was undertaken at the time of weaning by dipping the mice in a 2% malathion emulsion and then placing them in clean animal jars. The dipping was deemed superior to the litter treatment as the jars and litter were changed every 2 weeks, thus removing any insecticide residue that might have been left as the result of a litter treatment.

Individuals were selected at random and sacrificed at 24 hours, 48 hours and at weekly intervals up to the fourth week for evaluation of the control. Compete control of the infestation was effected immediately and at no time during this period was there any indication of a build-up or reinfestation.

[1] Accepted for publication June 24, 1957.

[2] Civilian Medical Entomologist, Third U. S. Army.

[3] DeterMination was verified by Dr. E. W. Baker, Entomology Research Division, U. S. Department of Agriculture.

An Emergency Summer Treatment of Pear Leaf Blister Mite[1]

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In 1956 the pear leaf blister mite (*Eriophyes pyri* (Pgst.)) threatened to cause unusually severe damage to pears in some areas of the Pacific Northwest. This threat was due in part to omission of a dormant spray because of the condition of the trees. Many of them had suffered severe winter injury which might have been aggravated by dormant spray materials. It was also due to a gradual increase in blister mite populations, which has been going on for several years, possibly because of changes in spray practices.

One such change may be the use of parathion in the prepink spray to replace lime-sulfur, alone or with oil, as a dormant treatment. This has been a welcome change to many growers, especially those who do not have water readily available until shortly before bloom. Further, many pests are controlled with parathion at least as well as with lime-sulfur, but not the blister mite. Parathion is a depressant, but certainly not a control, for this mite (Lanchester 1954). Under normal conditions a depressant has given adequate control.

Another change that may have permitted blister mite populations to increase, although less clearly evident, has been the swing from parathion to malathion for control of pear psylla (*Psylla pyricola* Foerst.). This is also a worthwhile development for growers who do not like to use parathion, but the low dosage of malathion used to control the pear psylla has little effect on the pear leaf blister mites within the blisters.

Whatever the causes, in the spring of 1956 entire pear orchards showed such heavy blister mite damage to the unfolding buds that owners sought some means of saving their trees. They had no hope for the crop. It was too late for dormant sprays. The only possibility was demeton. Lanchester (1954) had shown that the equivalent of 1 quart of the available (21%) formulation per 100 gallons would control mites within the blisters without injuring pear foliage. These tests had been on nonbearing trees and did not give any information on the effect of the material on the fruit. However, demeton had just received label approval for use on bearing pear trees. The Washington State spray recommendations (1956) warned of possible damage to pears at strengths in excess of one-half pint per 100 gallons. The label issued by the manufacturer limited application to pear foliage to not over three-fourths pint per 100 gallons.

In the absence of any alternative recommendation, demeton was widely used in the Yakima, Wash., area in the late spring and early summer of 1956. The quantity of 21% demeton emulsifiable concentrate per 100 gallons varied with the degree of infestation, as follows:

[1] Accepted for publication June 26, 1957.

(1) All trees generally infested throughout, with damage at time of spraying sometimes reaching an estimated 70% of the leaf surface, the crop believed to be a total loss—1 quart (6 quarts per acre).

(2) All trees infested but not all parts of every tree—½ to ⅔ quart (3 to 4 quarts per acre).

(3) Only an occasional infested terminal—½ pint (3 pints per acre).

The results of the treatments were remarkable. No damage to either leaves or fruit due to demeton was observed or reported. A satisfactory crop of good-quality fruit matured. A not excessive drop together with normal thinning removed fruit that at the time of treatment was damaged beyond recovery. Much of the earlier disfigurement must have disappeared, for only one report of blister mite injury, on harvested pears sprayed with 3 quarts or more of demeton per acre, was received, and this was in one small block of very young trees in which some fruit damage persisted until harvest. The treated trees made normal or better-than-normal growth.

Although many leaves on the treated trees had large blistered areas, they did not lose their green color. The blistered areas continued to be more opaque than the undamaged tissue, but generally did not die and turn brown as on untreated leaves. Still-folded and newly spread, rapidly growing leaves show the damaged areas by purple coloration, which is lost as the leaf matures. For some time after treatment these purplish areas with the characteristic openings on the under surface continued to appear, but they disappeared after a few days.

Blister mites in all stages could be found in all the plots if enough material was dissected under magnification. This indicates that either summer treatment of pears with demeton, even at 1 quart per 100 gallons of spray, does not eradicate an infestation or reinfestation occurs more rapidly than is generally believed. With the high cost for material, and the dislike that some operators have of applying it, its use must be considered an emergency treatment for pear leaf blister mite control.

Other materials that are now, or soon will be, available show promise for summer treatment of the pear leaf blister mite. More work must be done before they can be recommended for such use.

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N-Methyl-1-Naphthyl Carbamate as a Killing Agent for the Periodical Cicada[1]

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In late May 1957, Castillo Graham and E. R. Krestensen of the University of Maryland Field Station in Hancock, Md. obtained high kills of the periodical cicada, *Magicicada septendecim* (*L.*), with sprays of the new insecticide N-methyl-1-naphthyl carbamate,[2] named Sevin by the manufacturer. Graham and Krestensen used a wettable powder formulation containing 50% N-methyl-1-naphthyl carbamate at the rate of 2 pounds per 100 gallons in high volume sprays and at 6 pounds per 100 gallons in low volume mist concentrate sprays.

To broaden the information concerning Sevin as a killing agent for the periodical cicada, two plots of infested apple trees on the farm of John B. Peters, near York Springs, Pa., were sprayed on June 7, 1957. The wettable powder formulation was applied to separate plots at the rates of 1 pound and 2 pounds per 100 gallons, respectively, in high volume sprays with a spray gun.

Approximately 4 hours after application of the sprays, living but incapacitated cicadas were collected from the grass beneath the trees in the sprayed plots and placed in marked hardware cloth cages. A similar number of cicadas were collected from an untreated section of the orchard at a considerable distance from the sprayed plots, and also caged as control specimens.

Twenty-four hours after the sprays were applied, the caged cicadas were examined. The results were as follows: (1) all 63 of the cicadas sprayed with the 2-pound concentration of Sevin wettable powder were dead; (2) 62 of the 63 cicadas sprayed with the 1-pound concentration were dead; (3) only 9 of the 65 cicadas in the control sample were dead.

These results indicate that as far as immediate kill by contact is concerned a concentration of 1 pound of Sevin wettable powder in 100 gallons of high volume spray will probably give almost as high percentage kill of the periodical cicada as a concentration of 2 pounds. These results, however, do not measure the residual killing power of N-methyl-1-naphthyl carbamate against this pest.

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[2] Manufactured by Union Carbide Chemicals Company.

An Improved Technique for Determining Pink Bollworm Infestations in Cotton Samples[1]

CLYDE A. RICHMOND and C. N. HUSMAN, *Entomology Research Division, Agr. Res. Serv., U.S.D.A.*[2]

A rapid and economical method has been developed for determining pink bollworm (*Pectinophora gossypiella* (Saund.)) infestations in seed cotton, cottonseed, and crop debris.

Prior to 1955 the common technique used in both research and survey work was hand examination of the infested material; cotton-seeds were cut and individually examined. This method was tedious and time-consuming, and consequently a rather expensive operation. Furthermore, its accuracy was doubtful, particularly when the samples were lightly infested. For example, in gin tests to determine the kill from the various machines used in the process of ginning, there was the possibility of recording the injured larvae as alive, when in reality many would die of injury. Therefore, since pink bollworm research has expanded in recent years, it was desirable to develop a more functional and economical technique. At the Brownsville, Texas, laboratory a temperature- and humidity-controlled room was set up to meet the conditions necessary to cause the larvae to pupate and emerge as soon as possible.

A room was constructed approximately 20 by 16 by 8 feet and insulated in order to maintain the temperature and humidity at the desired level. A lighting system was installed consisting of overhead 15-watt ultraviolet lights, commonly called black lights, about 4 feet apart, to attract pink bollworm moths into the trap bottles, as they are positively phototropic to ultraviolet rays. The temperature was controlled easily with a regular commercial thermostat, as it is not critical and can vary a few degrees one way or the other. The humidity was controlled by using a standard humidistat. A high relative humidity is difficult to obtain and maintain. It can be obtained in several ways. At this laboratory a combination of evaporation and humidification by a nozzle system such as used in greenhouses proved satisfactory. The samples were also moistened when placed in the cages.

Several types of emergence cages can be used in this type of

[1] Accepted for publication June 28, 1957.

[2] In cooperation with the Texas Agricultural Experiment Station.

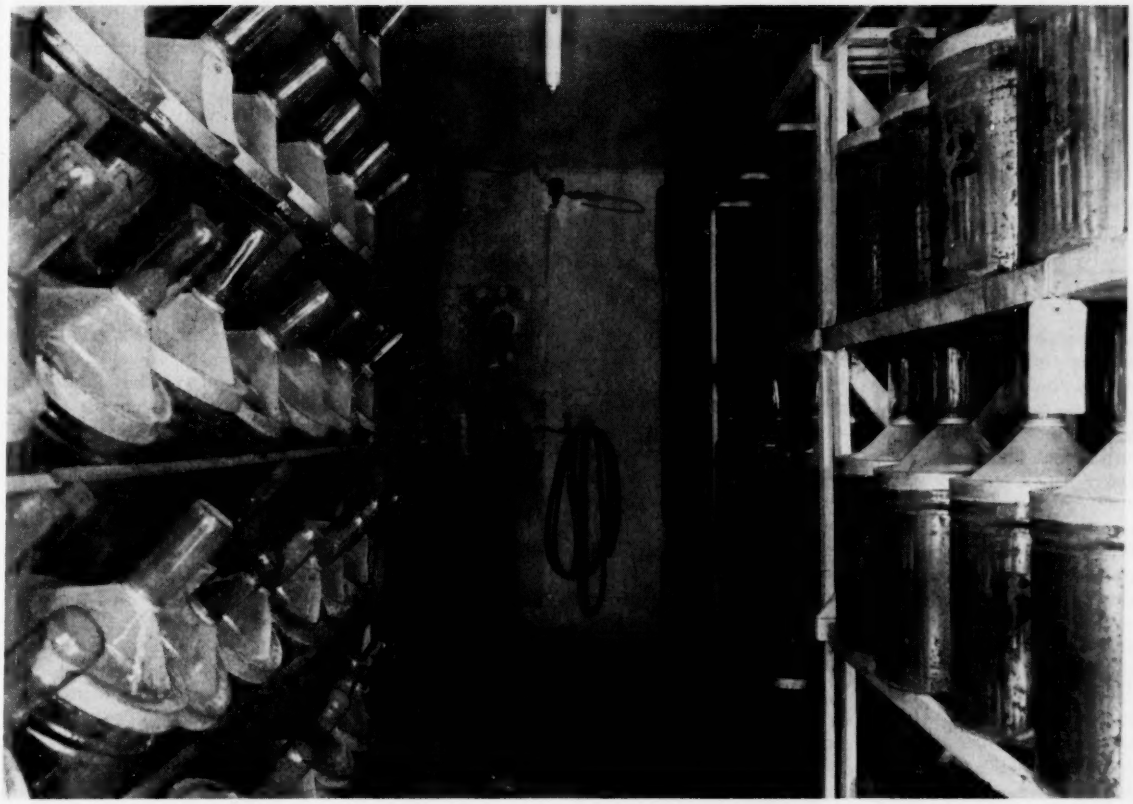

FIG. 1.—Room for holding moth-emergence cages. Left, rack with cages in slanting position; right, rack with cages in vertical position

work. The 4-gallon cans commonly used in frozen-food plants were found to be very satisfactory. These cans were fitted with cone tops made of 18-mesh screen wire. At the apex of the cone a trap bottle was fitted or screwed on in the usual manner, as on outdoor hibernation cages (Shiller 1946). The cans were placed on movable horizontal racks made of 1- by 2-inch boards that would accommodate as many as 132 cans per rack. The emergence room at this laboratory has a capacity of approximately 600 cans. A more satisfactory rack was designed to hold the cans in a slanting position, and this gave uniform exposure to the ultraviolet lights, better visibility, and easier manipulation (Fig. 1).

The daily means of the hygrothermograph records for a period of 3 months were as follows: Maximum temperature 89° F., minimum temperature 83° F., maximum relative humidity 75%, minimum relative humidity 57%. It is imperative that the cottonseed and other crop debris that make up the samples be kept moist. These conditions break the diapause and stimulate pupation and emergence of the adults. Present records indicate that when these conditions can be maintained approximately 95% of the pink bollworm larvae will pupate and emerge within 90 days.

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Effects of Soil Insecticides on Full-Grown Corn Earworm Larvae, Pupae, and Adults[1]

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Tests were conducted in central Illinois in 1955 to determine whether residues of insecticides applied to the soil in early May would have any affect on larvae of the corn earworm (*Heliothis zea* (Boddie)) that enter the treated soil or on resultant pupae or adults in late July or August.

Aldrin, heptachlor, and dieldrin at dosages of 4.9, 4.1, and 2.4 pounds per acre, respectively, were sprayed broadcast in emulsions over the surface of the soil in plots 40 feet wide and several hundred feet long. One plot was left untreated as a check. All the plots were disked to a depth of 4 inches immediately after the spraying, and the field was planted to dent corn a week later.

Soil samples were taken from the plots immediately after the application and twice later in the season. A composite sample consisted of 50 cores 2 inches in diameter and 4 inches deep from each plot. Three subsamples from each composite sample were analyzed for total chlorine.[2]

On July 27 four lots of 12 full-grown corn earworm larvae were placed in each plot and allowed to burrow into the ground to pupate. Each lot was confined within an area 1 foot square. To keep the larvae from wandering away, each one was covered with a 2-ounce salve tin minus the lid and the edge pressed lightly into the soil. A few kernels of corn were supplied for food until the larvae entered the soil. A 1-cubic-foot screen-wire cage

[1] Accepted for publication July 1, 1957.

[2] Samples were analyzed by Jack E. Fahey, of the Entomology Research Division.

Table 1.—Results of exposure of full-grown corn earworm larvae, pupae, and adults to soil treated with insecticides on May 5.

Insecticide	Pounds per Acre	Total Chlorine (P.P.M.)			Per Cent of Live Pupae on on Aug. 9	Per Cent of Adults Emerged by Aug. 18	Per Cent of Dead Forms on Aug. 18
		May 5	June 22	Aug. 15			
Aldrin	4.9	3.12	1.07	0.89	83	50	5
Heptachlor	4.1	2.84	1.67	1.13	83	53	11
Dieldrin	2.4	1.16	0.72	0.41	92	61	8
Untreated	—	—	—	—	58	67	3

was then placed over each group of 12 inverted tins to protect the larvae and to trap any moths that emerged from pupae formed in the soil. Each inverted tin was examined once, and dead larvae found on the soil surface were replaced with living ones.

On August 9 the soil under one cage in each plot was excavated to determine the number of living pupae. On August 16 and 18 the other three cages in each plot were examined for adults and on August 18 the soil under them excavated to determine the status of any forms remaining in the soil.

The results of these tests, shown in table 1, indicate that aldrin, dieldrin, or heptachlor applied to the soil in cornfields at planting time at recommended rates, or even somewhat higher, would have little effect on mature earworm larvae entering the soil after leaving the ears or on pupae or adults developing from such larvae.

There was a smaller percentage of live larvae in the soil in the untreated area than in the treated areas, probably owing to their destruction by the large number of ants present in the untreated area. There were practically no ants in the treated areas, and other experiments have shown that all the insecticides, even when applied at much smaller dosages, greatly reduce ant populations in cornfields.

A New Systemic Insecticide for Cotton Insects[1]

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In the screening program being conducted at College Station, Texas, to discover new compounds effective against insects and spider mites on cotton, three new systemic insecticides were discovered during 1953 (Ivy *et al.* 1954). All these compounds were 0,0-dialkyl S-alkyl mercaptomethyl dithiophosphates–Am. Cyanamid 12008, 12009, and 12013. In a continuation of this program during 1954, in which more than 200 new compounds were screened, another promising compound—Thimet—closely related to the three discovered in 1953, was brought to light. These compounds are systemically effective against certain chewing cotton insects.

To be effective systemically against chewing insects, such as the boll weevil, most compounds must be applied to the soil or to the seed, as they do not translocate efficiently when applied as sprays in the conventional manner (Ivy 1953). Seed treatment requires considerably lower dosages than soil applications, although the period of effectiveness may not be so long (Ivy *et al.* 1950). An activated-carbon carrier impregnated with equal parts of the insecticide has proved to be an effective formulation for treating cottonseed for systemic action. Much higher dosages of the systemic compounds formulated in this way are tolerated by the seed and seedling plants than when liquid dip is used, and the threatments are more persistent (Ivy 1952). Machine-delinted cottonseed will adsorb from 8 to 12 pounds of the impregnated carbon dust per 100 pounds of seed, and this amount will adhere tenaciously. However, this treatment is not suitable for acid-delinted or other smooth seed.

Some of the insecticides that kill boll weevils and other chewing insects placed on seedling cotton plants grown from seed receiving them in systemic treatments are parathion, methyl parathion, and para-oxon (Ivy *et al.* 1954). Unfortunately, however, each of these materials inhibits germination, and injures seedling plants when applied at dosages required for control. Demeton and a number of related thiophosphates are less effective against chewing insects and are also phytotoxic when used in this way.

The procedures used in these tests have been described (Ivy 1952). From 60 to 200 adults of the boll weevil, *Anthonomus grandis* Boh., and from 200 to 300 cotton aphids, *Aphis gossypii* Glov., or spider mites, *Tetranychus tumidus* Banks, were used in each test. Ten newly hatched larvae of the salt-marsh caterpillar, *Estigmene acrea* (Drury), and the bollworm, *Heliothis zea* (Boddie), and 30 to 60 flower thrips, *Frankliniella tritici* (Fitch), or onion thrips, *Thrips tabaci* Lind were tested. In the tests with the cotton leaf perforator, *Bucculatrix thurberiella* Busck, the test plants were grown in a section of the greenhouse infested with the test insect. All materials were applied to cottonseed before planting at the rate of 4 pounds of insecticide per 100 pounds of seed.

Systemic effectiveness was assumed to have disappeared when less than 75% net mortality could be obtained during the test period, or when leaf mines of the cotton leaf perforator were found. The test period was 5 days for the boll weevil, salt-marsh caterpillar, and bollworm, 3 days for the cotton aphid and spider mite, and 2 days for onion and flower thrips.

The systemic insecticidal action of Thimet persisted longer than that of the other three compounds. This was particularly true in case of boll weevils, thrips, and cotton leaf perforators. Thimet is thus an extremely promising new systemic insecticide and should be extensively tested against cotton insects.

In tests designed to determine the top dosage of Thimet that could be applied to seed without injury, it was found that under laboratory conditions no reduction in germination or loss in stand resulted from 32 pounds per 100 pounds of seed. Small necrotic lesions appeared on leaves of plants grown from seed treated at the rates of 16 and 32 pounds, but no injury of any kind was apparent in seed treated at 8 or 4 pounds per 100 pounds of seed.

When applied as a spray in the conventional manner, Thimet was effective against the boll weevil by contact action. Two pounds of the technical material per acre gave satisfactory control in these tests, but weevils placed on the treated plants 1 day after the application were not killed. It is apparent, therefore, that insofar as boll weevil control is concerned, insecticidally significant quantities of the compound are not absorbed by the plant following spray application to cotton foliage. In this respect the compound behaves in exactly the same manner as

[1] Submitted for publication March 30, 1955, but did not reach the editor's desk until July 19, 1957.

[2] In cooperation with the Texas Agricultural Experiment Station.

Table 1.—Duration of effectiveness (weeks) of four systemic insecticides against various cotton insects.

Compound	Boll Weevil	Cotton Aphid	Spider Mite	Salt-Marsh Caterpillar	Bollworm	Flower Thrips	Onion Thrips	Cotton Leaf Perforator
Thimet	7	9	9	2	0	8	7	9
Am. Cyanamid 12008	5	9	9	2	0	3	6	5
Am. Cyanamid 12009	3	3	5	0	0	4	6	4
Am. Cyanamid 12013[a]	0	1	2	0	0	0	6	1

[a] 0,0-diisopropyl S-isopropyl mercaptomethyl dithiophosphate.

parathion, methyl parathion, and para-oxon (Ivy *et al.* 1954).

Prolonged (2 weeks or more) aphid and spider mite control resulted from foliage application of Thimet at 0.25 pound per acre. In these tests the compound was slightly less effective than demeton similarly applied.

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Effect of Several Insecticides on the English Red Worm[1]

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Economic entomologists are concerned mainly with increasing crop yields by controlling insect pests with insecticides, and many of them give little consideration to the effects of the insecticides on soil fauna. One of the important soil organisms is the earthworm.

Much controversy about the benefit of earthworms to field crops exists. However, it has been shown that they increase yields (Hopp *et al.* 1949). Their beneficial effects are increased aeration and infiltration (Evans 1948), maintenance of soil structure (Dutt 1948), and concentration of nutrients making them better available for crop growth (Lunt *et al.* 1944).

Hyche (1956) reported that earthworms were killed by certain dosages of Metacide, malathion, and parathion used to treat earthworm cultures for mite control. Morrison *et al.* (1954) found that most insecticides were toxic to earthworms if applied to the area of feeding, but soil treatments with aldrin, heptachlor, and dieldrin at the rate of 10 pounds per 6-inch acre had no measurable effect.

Laboratory investigations were undertaken at Florence, S. C., in 1956 to determine the effect of 10 cotton insecticides upon the English red worm, *Eisenia* sp., probably *foetida*.[2] This is the species commonly raised commercially for fishing worms.

Two experiments were conducted. In the first experiment the rates used were based on 10 applications at the maximum recommended dosages for seasonal cotton-insect control, and in the second the dosages required to kill 50% of the test species were determined. In both experiments Marlboro sandy loam was used as the test medium. A culture of the test species was maintained in organic soil with peat moss added. All insecticide-rate calculations were made on the assumption that a 6-inch depth of mineral soil over 1 acre weighs approximately 2 million pounds.

In the first experiment 33 two-gallon glazed ceramic pots were arranged in a randomized-block design. Thirty-three kilograms of dry soil, the calculated amount of insecticide in dust form for each treatment, and 25 grams of corn meal for food were mixed for 20 minutes with a roller mixer; then 250 ml. of water was added and thoroughly incorporated by hand. The mixture was divided into equal parts and placed in separate pots to make three replicates for each treatment. Ten earthworms were placed in each pot. Similar moisture levels were maintained by spraying the soil surface of each pot for an equal period with a 6-X nozzle attached to a water hose. Two days and 1, 4, and 8 weeks after treatment the soil was poured from each pot and the number of live earthworms determined.

As shown in table 1, none of the treatments resulted in a sig-

Table 1.—Survival of English red worms after 2 months' exposure in soils treated with several organic insecticides.

Treatment	Pounds of Toxicant per Acre	Per Cent of Adults Surviving	Average Number of Young
Check	—	100	10.3
Dieldrin 1.5%	5.0	100	3.3
Endrin 2%	5.0	100	4.0
BHC 3% g.i.	5.0	96	9.0
Guthion 2.5%	5.0	93	1.0
Heptachlor 2.5%	7.5	93	0
DDT 10%	15.0	93	18.0
Malathion 5%	7.5	90	13.3
Aldrin 2.5%	7.5	83	1.6
Toxaphene 20%	30.0	76	0

nificant mortality after 2 months' exposure of the adult worms. The number of young worms found varied, but an analysis revealed no significant difference in these numbers. However, it should be noted that no young worms were found in the heptachlor and toxaphene treatments. These results indicate that harmful effects would be unlikely following normal usage of these insecticides. No allowance was made for normal breakdown of the insecticides or loss through leaching.

In the second experiment 3.3 kilograms of dry soil, the calculated amount of insecticide in dust form for each treatment, and 7.5 grams of corn meal for food were mixed as in experiment 1; then 50 ml. of water was added and thoroughly incorporated by hand. Three replicates were made by dividing this mixture into equal parts and placing them in ½-pint ice-cream containers. Five earthworms were placed in each container, and a lid was put on each. This was essential, since at the high rates used the worms frequently would not enter the soil and sought to escape.

[1] Accepted for publication July 19, 1957.

[2] G. E. Gates, of the U. S. National Museum, made this determination.

Table 2.—Effect on English red worms of soil applications of insecticides at different rates, Florence, S. C., 1956.

Insecticide	Pounds of Dust per Acre	Per Cent Net Mortality	LD-50 at 96 Hours: Pounds of Dust per Acre
Chlorinated hydrocarbons:			
Dieldrin 1.5%	32	0	183.2
	64	20	
	128	33	
	256	100	
Aldrin 2.5%	64	0	170.3
	128	13	
	256	93	
Heptachlor 2.5%	32	6	163.0
	64	8	
	128	27	
	256	80	
Chlordane 5%	32	0	100.0
	64	46	
	128	40	
	256	79	
BHC 3% gamma	32	0	60.0
	64	60	
	128	100	
Phosphorus compounds:			
Malathion 4%	16	0	37.9
	32	44	
	64	68	
Guthion 2.5%	8	12	12.2
	16	76	
	32	100	

Moisture was maintained by pouring an equal amount of water into each container when it was needed. The temperature was maintained at 75° F. After 4 days the soil was poured from each container and the number of live worms determined. The earthworms were considered dead when no response was observed after probing with a pencil.

To evaluate the relative toxicity, the mortality data were subjected to probit analysis as described by Finney (1952). The 96-hour median lethal dose for each insecticide is shown in table 2. The organic-phosphorus compounds were more toxic and probably create a much greater hazard to the English red worm than the chlorinated hydrocarbons. Guthion was the most toxic compound tested and was approximately three times as toxic as malathion, the other phosphorus compound. BHC was the most toxic chlorinated hydrocarbon, possibly owing to its fumigant action, but it was less toxic than malathion.

These tests indicate that many years of consistent application of chlordane, heptachlor, aldrin, or dieldrin would be necessary to endanger populations of English red worms. Tests with up to 256 pounds of DDT and 1024 pounds of toxaphene or endrin per acre gave highly erratic results, but extremely high rates would be necessary to decimate populations of the English red worm.

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Susceptibility of the Boll Weevil in South Carolina to Several Insecticides[1]

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In 1955 Roussel and Clower reported that in certain areas in Louisiana the boll weevil (*Anthonomous grandis* Boh.) had apparently developed resistance to chlorinated hydrocarbon insecticides. Additional data obtained in 1956 (Roussel *et al.*) confirmed the 1955 findings. To determine if such resistance had developed in South Carolina and to obtain data that might later be used as a comparison if resistance is suspected, laboratory toxicity tests were undertaken at Florence in the spring of 1956.

It was realized at the outset of this study that weevils from areas that had consistently received chlorinated hydrocarbon insecticides for several years might be more tolerant of these insecticides than weevils from areas that had received only small amounts at infrequent intervals. Some insecticides are used wherever cotton is grown in the State, but in the coastal area of Georgetown County very little is grown and very little insecticide is used. Consequently arrangements were made to grow in this area a field of cotton to which no insecticide would be applied and which would serve as a source of susceptible weevils to compare with those from other areas.

Early-season tests were conducted with overwintered weevils hand-picked from seedling cotton in the vicinity of Florence, and later tests with weevils reared from punctured squares and bolls collected from different areas in the State. The infested material was placed in screen-covered rearing trays and held in the laboratory at approximately 80° F. until emergence was complete. The emerged weevils were removed daily and fed for 2 days on fresh cotton squares before they were utilized in the toxicity tests.

The insecticides were applied at 1-microliter dosages in acetone to the dorsal surface of the weevils on the suture between the metathorax and the elytra. To obtain a dosage-mortality curve, a series of dilutions of a given insecticide was applied to different groups of weevils, each dilution being twice the strength of the preceding one. Approximately 50 weevils, usually as 5 replications of 10 weevils each, were treated with each dilution. After treatment the weevils were placed in cages containing fresh squares for food and held for 48 hours in the laboratory at 78 to 85° F. and a relative humidity of 75 to 90%. Before treatment the weevils were weighed in groups of 10 or 20, the average weight determined, and the dosage of insecticide was later computed using the method of Finney (1952).

A limited number of overwintered weevils from the vicinity of Florence was available for the early-season tests. The LD-50 obtained with endrin against these weevils was 0.00167 mg. per gram, indicating a high degree of susceptibility to this insecticide.

The LD-50's obtained with the recommended insecticides

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[2] In cooperation with the South Carolina Agricultural Experiment Station.

Table 1.—Median lethal dosage (milligrams per gram) of endrin and Guthion to boll weevils from different sources in South Carolina.

Source of Weevils	Endrin			Guthion		
	George-town	Florence	Darling-ton	George-town	Florence	Darling-ton
Summer:						
Square-reared	0.00711	0.0087	1.160	0.00163	0.00183	0.00208
Fall:						
Square-reared	.000472	.2087	.213	—	—	—
Boll-reared	.00314	.00488	—	—	.00191	—
Field-collected	—	—	1.173	—	—	—

against weevils reared from squares collected in Florence County during the summer months are given below.

	Milligrams per Gram
Chlorinated hydrocarbon insecticides:	
Endrin	0.0087
Aldrin	.0183
Dieldrin	.0206
BHC (gamma)	.0232
Heptachlor	.0281
Chlordane	.294
Toxaphene	.543
Phosphorus insecticides:	
Guthion	.00183
Methyl parathion	.0105
EPN	.0161

Since in this area considerable amounts of chlorinated hydrocarbons had been used for several years, the LD-50's obtained with these insecticides may not be representative of a wholly susceptible population. However, they will serve as a basis on on which to determine whether a high resistance later develops, since satisfactory control was obtained with all of them during 1956.

In July the Coker Pedigreed Seed Company in Darlington County reported that 14 applications of endrin had failed to control the boll weevil in one of its cotton fields. To find out whether resistance was the cause of this failure, punctured squares were immediately collected from this field and weevils reared from them for comparison with weevils reared from squares collected in Florence and Georgetown Counties. In these tests 163 times as much endrin was required to kill 50% of the weevils from Coker farm as to give the same kill of weevils from Georgetown County and 133 times as much as the Florence weevils. These results confirmed the suspicion of resistance of the Darlington weevils to endrin.

The results obtained with endrin and Guthion against weevils from different sources, presented in table 1, clearly show the degree to which the Darlington County weevils are resistant to endrin. Tests with the fall square-reared weevils from Florence County also indicate that resistance may be a fact at that location; however, this may be seasonal variation, as reported by Rainwater & Gaines (1951). Comparison of the LD-50's of square- and boll-reared weevils is not possible, as the material was obtained from different locations. The Guthion data indicate no trend toward resistance at any location.

Late-season tests were conducted to compare the effectiveness of BHC against weevils from the resistant area in Darlington County with that against similar weevils from Florence and Anderson Counties. The LD-50's obtained in these tests are presented in table 2. Upward of 20 times as much of the gamma isomer was required to kill 50% of field-collected weevils from Darlington County as those from Florence and Anderson Counties, but there was little difference in the susceptibility of late square-reared weevils from Darlington and Florence Counties. When these results are compared with those obtained with summer square-reared weevils, it is evident that the fall weevils are harder to kill. This seasonal variation has been reported by Rainwater & Gaines (1951).

Table 2.—Median lethal dosage (milligrams per gram) of BHC to fall boll weevils from different sources in South Carolina.

County	Field-Collected Weevils	Square-Reared Weevils
Darlington	1.507	0.0778
Florence	0.737	.0519
Anderson	.0668	—

Late in the summer of 1956 the Food and Drug Administration reported that certain combinations of the organic phosphorus insecticides showed much greater toxicity to mammals than would be expected from a simple additive effect. In order to determine if this "potentiation" occurs in the boll weevil, topical-application tests were conducted with EPN, malathion, and a 1:5 mixture of EPN and malathion. The respective LD-50 values were found to be 0.0429, 0.00979, and 0.0207 mg. per gram. The combination was only slightly more effective than the additive effect of the two components.

During the summer, reports were received from Mississippi that a 5:2 mixture of malathion and endrin was highly effective against endrin-resistant weevils. A test with this mixture was conducted on field-collected endrin-resistant weevils from Darlington County. The LD-50 value was found to be 0.0699 mg. per gram. When this is compared with the value found for endrin alone (table 1), it is seen that the ratio of effectiveness is approximately 16:1 in favor of the mixture.

To determine how far the resistant area in Darlington County had spread from the field in which resistance was observed earlier, tests were conducted late in the season with square-reared weevils from this field and from fields 0.3, 0.6, and 1.2 miles away. The respective LD-50 values were found to be 0.213, 0.0612, 0.0287, and 0.0350 mg. per gram. Thus, weevils migrating from the resistant population were having some influence on the population in the field 0.3 mile distant, but not on those in the fields 0.6 and 1.2 miles distant.

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Seed Treatments of Wheat for Control of the Great Basin Wireworm[1]

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The Great Basin wireworm, *Ctenicera pruinina noxia* (Hyslop) is a persistent pest of small grains in the dry-farming areas of the Snake and Columbia river drainage systems. Lane (1931) has described the biology of this insect, and the cultural practices that help to minimize damage.

Experiments were undertaken to control this insect by the most economical means possible. Soil treatments with lindane showed some promise, but were not tested further because they were not economical in a summer fallow economy. Seed treatments offered an ideal way of solving the problem, because the insecticides could be added when fungicides for controlling smuts were applied by the slurry treatment. To be effective, insecticides applied to fall-planted wheat must remain sufficiently toxic the following spring when wireworm damage occurs.

The insecticides were generally applied as wettable powders (Tables 1–2). These were thoroughly roll-mixed in gallon jars by the mixer designed by Chao & Roberts (1954) with sufficient water to slightly dampen the seed. In the tests conducted during the fall of 1955 and during the spring of 1956, an 0.3% aqueous solution of methyl cellulose solution was substituted for water, as a sticker. Better coverage at the higher levels was possible with wettable powders of 50% or greater concentration, than those of a lower concentration.

In addition to the field tests for determining protection against wireworms, treated seed was tested for germination to determine phytotoxic effects. These tests were performed by incubating the treated seed for 1 week at 28° C. between damp blotters, or damp sand, and subsequently examining them for development. The germination tests, table 1, showed that viability was not significantly reduced by most of the materials tested. Lindane caused general stunting of the sprouts but did not prevent germination. The same effect occurred to a lesser extent with so-called "safened lindane," a formulation containing Aroclor 5460. The germination tests using moistened blotters probably accentuated any possible injury. Extensive stand reduction was not noted with seed planted in soil, when lindane was applied at the rates tested here. Dr. Laurence H. Purdy, U.S.D.A. Plant Pathologist at the State College of Washington, reported to the authors that extensive reduction of germination occurred with commercial lindane-fungicide preparations at rates of lindane no higher than those tested here, though he stated that considerable variation in this effect appeared due to environmental conditions. The fungicides tested here were applied at the manufacturer's recommended dosages.

Field plots were planted at Lind, Washington, and were designed to accentuate wireworm injury. Seeding was at 30 pounds to the acre (about half the usual seeding rate), and as late in the fall as feasible in order to have smaller and weaker seedlings at the time of wireworm injury in the spring. Plots were laid out with a random design, and consisted of three or four replications in each test. Each replication was four rows wide and up to 100 feet long. Counts were made in the spring when maximum wireworm injury was visible. The total number of healthy and wireworm-damaged plants were counted from 72, 68, or 48 feet (dependent on the season) in the two center rows of each replicate.

The materials chosen, for both fall- and spring-planted wheat, were aldrin and heptachlor. Lindane was also effective, but is not recommended because of possible phytotoxic effects. Lindane, "safened" with Aroclor 5460, was reduced in its effectiveness for controlling wireworms. Dieldrin was effective, but more expensive than aldrin or heptachlor. Tests with spring wheat indicate that Guthion and Thimet have limited effectiveness. Dead wireworms and a few dead larvae of false wireworms (*Eleodes* species, family Tenebrionidae) were commonly observed in plots treated with effective materials, indicating mortality and not simply repellancy. The fungicides Ceresan M (N-(ethylmercuri)-*p*-toluenesulfonanilide) and PMA (phenylmercury acetate) did not appear to alter the effective insecticides tested. It was not determined whether the insecticides affected fungicidal action.

Yield data were obtained from all plots for the 3 years. Despite reduction of the stand by as much as 20%, no significant dif-

Table 1.—Germination tests on two varieties of wheat. Pullman, Wash., 1953–55.

Treatment	Active Ingredient (Oz./Bu.)	Per Cent Strong Sprouts: Immediate Test	Per Cent Strong Sprouts: After Storage[a]
Fall 1953—Rio Wheat (without fungicide)			
None	0	92.3	
Aldrin	2.0	94.3	
Dieldrin	2.0	93.3	
Heptachlor	1.0	92.5	
Isodrin	1.0	95.0	
Lindane	0.5	0	
Fall 1954—Rio Wheat (with fungicide)			
None	0	99	
Ceresan M (fungicide) alone		99	
Aldrin	2.0	95	
Dieldrin	2.0	97	
Dipterex	3.0	94	
Heptachlor	2.0	92	
Lindane	0.5	90	
	1.0	54	
	2.0	0	
Lindane+Aroclor 5460[b]	0.5	89	
	1.0	47	
	2.0	3	
Spring 1955—Federation wheat (with fungicide)			
None		85	76
PMA (fungicide) alone		90	80
Aldrin	2.0	94	61
Am. Cyanamid 12008	2.0	86	61
Dieldrin	2.0	92	64
Dipterex	2.0	92	72
Heptachlor	2.0	94	68
Lindane	2.0	5	4
Thimet	2.0	86	61
Fall 1955—Rio wheat (without fungicide)			
None		94	
Aldrin	2.0	93	
Am. Cyanamid 12008	2.0	94	
Bayer 16259	2.0	94	
Guthion	2.0	95	
Heptachlor	2.0	94	

[a] Test was begun 78 days following treatment, storage was in closed bottles at room temperature.

[b] A chlorinated polyphenyl produced by Monsanto Chemical Co. and formulated with lindane by Ethyl Corporation.

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Table 2.—Wireworm damage to treated seed. Lind, Wash. 1953–56.

TREATMENT[a] AND CONCENTRATION		ACTIVE INGREDIENT (OZ./BU.)	TOTAL PLANTS EXAMINED	PER CENT DAMAGED BY WIREWORM
Fall 1953—Wheat (without fungicide)				
None			1231	13.3
Aldrin dust	75%	2.0	1147	0.5
Dieldrin	50%	2.0	1125	2.8
Heptachlor	25%	1.0	1203	2.1
Isodrin	25%	1.0	1149	8.0
Lindane	75%	0.5	1152	5.2
Fall 1954—Rio Wheat (with fungicide)				
None			585	19.8
Ceresan M (alone)			672	9.4
Aldrin	20%	0.5	619	6.8
		1.0	682	4.4
		2.0	725	0.8
Dieldrin	20%	0.5	554	7.9
		1.0	657	5.9
		2.0	643	2.0
Dipterex	(Tech.)	0.5	661	10.9
		1.0	678	5.9
		2.0	655	14.8
Heptachlor	20%	0.5	657	2.3
		1.0	691	0.4
		2.0	577	1.2
Lindane	20%	0.5	671	2.2
		1.0	524	2.5
		2.0	209	0.5
Lindane +Aroclor 5460	20%	0.5	596	6.7
		1.0	716	1.3
		2.0	439	1.6
Spring 1956—Bart Wheat (with fungicide)				
None			853	3.2
PMA (alone)			940	4.7
Aldrin	75%	1.0	841	0
Am. Cyanamid 12008 (carbon)	50%	0.5	761	3.5
		1.0	897	0.8
		2.0	716	0.3
Bayer 16259	50%	0.5	881	0.6
		1.0	916	0.2
		2.0	953	0.4
Guthion	50%	0.5	851	0.8
		1.0	977	0.5
		2.0	859	0.2
Heptachlor	75%	1.0	989	0.2
Thimet (carbon)	50%	0.5	811	2.2
		1.0	936	1.0
		2.0	844	0.6

[a] Wettable powder was used except as noted.

ferences were noted between the yields of untreated plots and those where excellent control was obtained. This even yield was probably a consequence of a natural ability of the uninjured plants to send out supplementary tillers during favorable growing seasons. Probably seed treatment for control of the Great Basin wireworm is necessary only in areas of chronic, heavy damage, or where valuable seed stock is being propagated. The expense of treatment is slight, and the application method is compatible with the present practice of slurry treatment for smuts. Seed treatment for wireworms is a profitable insurance practice.

SUMMARY.—Twelve insecticides were tested as seed treatments for control of the Great Basin wireworm. From the standpoint of wireworm control, phytoxicity, and economy, aldrin and heptachlor gave the best control. Lindane was phytotoxic at higher levels under laboratory germination tests. Dieldrin was quite effective, but more expensive than aldrin or heptachlor. All effective insecticides apparently were compatible with the fungicides Ceresan M and PMA when applied at the manufacturers' recommended dosages. The treatment of seed fits in with the current practice of slurry-treating wheat for control of smuts.

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Tests with Thimet on Strawberries and Turnips[1]

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Control of mites and aphids on strawberries is often needed during the long harvest season, when most of the effective insecticides must be avoided to prevent excess residues on the fruit. A similar problem is encountered in the control of aphids on turnips. In 1956 preliminary tests were made in southern California to see if these pests could be controlled by early applications of the systemic insecticide Thimet. The results were so promising that they are summarized in this report.

The strawberry plants in this study were infested with the two-spotted spider mite (*Tetranychus telarius* L.), the green peach aphid (*Myzus persicae* (Sulz.)), the bean aphid (*Aphis fabae* Scop.), and an unidentified aphid of the genus *Macrosiphum*. The turnip plants were infested with about equal numbers of the turnip aphid (*Rhopalosiphum pseudobrassicae* (Davis)) and the green peach aphid.

Thimet was tested in 2% granules and in sprays made from an emulsifiable concentrate containing 4 pounds per gallon. One quart of this concentrate was used with 100 gallons of water to make a high gallonage spray and with 20 gallons of water to make a low-gallonage spray. Knapsack sprayers were used, an ordinary type to apply the high-gallonage spray and a high-pressure mist type to apply the low-gallonage spray.

TESTS ON STRAWBERRIES.—The strawberry plots were 30 by 3½ feet and consisted of two rows of plants on a ridge. They were arranged in eight randomized blocks. Thimet was applied on February 27 at ½ pound and 1 pound per acre by four methods: (1) in the low-gallonage spray to the foliage, (2) in the low-gallonage spray to the bottom of furrows made 4 inches to one side of each row and 4 inches below the crown of the plants, along the side of the irrigation furrows, (3) in the high-gallonage spray to the furrows, and (4) in the 2% Thimet granules to the furrows. The furrows were closed after treatment, and the field was irrigated on March 1. Counts were made of aphids surviving on five leaflets per plot on March 5, 14, 20, and 26 and on April 2, 16, and 23. The two-spotted spider mites were counted on April 16 and 23.

An analysis of the results showed that there was no significant interaction between dosages and methods of application. The

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Table 1.—Control of aphids and spider mites on strawberries with Thimet at 1 and ½ pound per acre applied by different methods.

Treatment	Aphids		Spider Mites	
	Number per Leaflet	Per Cent Reduction	Number per Leaflet	Per Cent Reduction
Average of two dosages				
Foliage spray, low-gallonage	0.7	97	0.0	99
Furrow spray:				
High-gallonage	.6	97	.1	98
Low-gallonage	2.5	87	1.4	79
Granules	10.7	46	3.9	40
L.S.D. at 5% level	2.5	13	2.3	36
Average of four methods				
Thimet dosage:				
1 pound	2.7	86	.8	87
0.5 pound	4.5	77	1.8	71
L.S.D. at 5% level	1.4	7	1.6	25

Table 2.—Control of aphids on turnips with Thimet granules and sprays.[a]

Thimet (Pounds per Acre)	November Applications		January Applications	
	Number per Leaflet	Per Cent Reduction	Number per Leaflet	Per Cent Reduction
In granules				
4	0.10	99	0.03	100
2	.47	93	.67	90
1	2.23	67	1.93	72
In sprays				
2	2.53	63	.57	92
1	3.33	51	2.20	68
0.5	4.10	40	2.60	62
L.S.D. at 5% level	1.85	27	1.85	27
Check	6.80	—	6.80	—

[a] January sprays applied to foliage; all other applications in furrow.

results in table 1 are therefore summarized by methods of application and by dosages.

This experiment indicates excellent control of both aphids and spider mites for 2 months by the application of Thimet sprays in the furrows near the strawberry plants, and this control was just as good as with sprays on the foliage. Applications of the granulated material were less effective. If further experiments confirm that good control can be obtained by applying systemic materials to the soil with no injury to the plants, it may be possible to work out a control schedule for these insects on strawberries without applying poisonous spray to the plants and fruit during the long picking season when residue hazard is such a serious problem.

Tests on Turnips.—The turnip plots were 25 by 3 feet and consisted of two rows of plants on a bed. They were arranged in six randomized blocks. Thimet was applied to half the plots on November 10, 1955, just prior to seeding. Furrows were made with a wheel hoe in the seed rows on the bed, the granules and sprays applied in these furrows and then covered with a rake, after which the seed was planted by the grower. The other half was treated on January 4, 1956, when the plants were in the six- to eight-leaf stage. The granules were applied in furrows made at one side of each plant row and then covered, and the sprays to the foliage at about 10 gallons per acre. Heavy rains occurred during the latter part of January. On February 9, just prior to harvest, the living aphids on five leaves per plot were counted, and the results are shown in table 2.

The high and medium dosages of the granules applied prior to seeding in November were very effective, and the soil applications made 2 months later were just as good. The furrow spray applied in November was inferior to all other methods and did not give adequate control. There was no significant difference between the foliage sprays applied in January and the granule treatments made at the same time at similar dosages.

Turnip samples from all the treatments were submitted to the American Cyanamid Company for analysis, and they were reported to contain a trace of Thimet, but less than 0.004 part per million.

BOOK REVIEW

Investigation of Virus Diseases of Brassica Crops, by L. Broadbent. Agric. Res. Council Rept. 14. vii+94 pp. illus. London. Cambridge Univ. Press. 1957.

These studies were made in England and Wales during 1950–55 with emphasis on virus spread in the field and on possible control measures. Brassica crops include turnip, swede, cabbage, savoy, kohlrabi, kale, rape, mustard, brussels sprouts, cauliflower, and sprouting broccoli. Cauliflower mosaic (ClMV) and cabbage black ring spot (CBRSV) viruses, both transmitted by aphids, are common. Turnip yellow mosaic and turnip crinkle viruses, transmitted by flea beetles and other biting insects, are locally severe.

Physical properties and symptoms of ClMV and CBRSV are given. Both viruses occur in strains. Symptoms of ClMV are more severe below 24° C., those of CBRSV above 24° C. Reactions of many crop varieties to ClMV are given. Hosts of ClMV and CBRSV are tabulated; the first named is confined to Cruciferae, the second infects plants of other families.

Aphids of 10 spp. transmit both viruses, 15 additional spp. transmit ClMV only, *Aulocorthum solani* transmits CBRSV only, and 9 spp. transmit neither. *Myzus persicae* and *Brevicoryne brassicae* are the only important vectors. Severe outbreaks of disease are usually associated with *B. brassicae*, but *M. persicae* is important in spring, transmitting from overwintering crops to seed beds, and sometimes during summer and fall. Preacquisition fasting did not improve vector transmission of ClMV; this virus survived 3 hours in feeding aphids, occasionally 24 hours in fasting aphids. Preacquisition fasting improved vector transmission of CBRSV; this virus was acquired in 10 minutes feeding, transmitted at once, retained 30 minutes in feeding aphids, 9 hours in starved ones. Annual and seasonal variation in prevalence of the two chief vector species and factors determining such variation are discussed.

ClMV is usually more prevalent in the field than CBRSV because the first is retained longer by vectors and occurs in high concentration in all young leaves, whereas CBRSV is present chiefly in older, lower leaves. Winged aphids, mainly responsible for virus spread, usually alight on the upper parts of a plant. Most spread is to plants within 8 paces of a virus source.

Spraying seed beds with various insecticides including systemics did not reduce incidence of disease, but barriers of plants (cereals) around seed beds reduced incidence 80%. Spread of Brassica viruses depends on proximity of one susceptible crop to another at particular times of the year. Planting blocks of similar age together, but well separated from older plants, is an important control measure.

Philip Brierley

EDITORIAL

Worldwide Compilations of Arthropods of Medical Importance[1]

Experience during the past two decades has brought rather forcefully to our attention the need for a better understanding of the worldwide distribution of arthropods of medical importance. Numerous unfortunate experiences in the past could have been either avoided or minimized had everyone been more aware of the local pest and disease problems in various areas of the world. These well documented catastrophies are ample proof of the need for more adequate data.

In 1950 the Office of the Quartermaster General asked Cornell University to accept a project which had as its primary purpose the compilation of a worldwide list of arthropods of medical importance, itemized country by country. In addition to the list, information was desired on the species that were vectors of disease, the seasonal activity of the species and any other pertinent biological data. In July of 1951, the final papers were signed, and Cornell embarked upon this huge project.

The project is a part of the military effort to become familiar with insect problems that may be encountered in overseas areas where troops may be stationed. This request was brought on by experience of World War II when it was found that one could not go to any one source and find needed information for medical logistics, and for guidance in planning the supplies necessary for troops. Actually as many now know, some very competent scientists made useful compilations during the war for specific areas.

One of our early problems with this project was not of an entomological nature. There seemed to be no listing of the countries of the world arranged in a fashion suitable for the zoögeographer. As one can readily imagine, political boundaries can have little significance to the zoögeographer. Yet, if the world is to be divided into small units it is certain that political boundaries must be utilized. As a consequence, a considerable effort was made to follow the political boundaries as recognized in 1950. In addition, the geographical listings include the political subdivisions such as state or province for most of the countries. The final listing, in which the countries are arranged alphabetically, includes over 350 categories. This list has now been used for about 5 years and has been found entirely satisfactory. With the basic list it is possible to place any country or island within the framework of the list.

In addition to the problem of preparing a geographical index, there was a need for some way of handling the very large amount of literature. After considering all the various possibilities of handling the citations the hand keysort cards were selected. Then a card was designed that would permit entries of all the necessary categories. Such a card was not found available, but the one which was finally used was a two-hole keysort card made by the McBee Keysort Company. The categories used in the subject index is actually a modification of one used several years ago by some of the workers in biology in the University of California at Berkeley.

Quite naturally since the subject matter deals with such a broad field, the individual index categories are necessarily broad. Workers in specialized and small fields will find many of the categories too broad for their needs. However, the system has been adequately flexible and yet also sufficiently selective to obtain rather quickly the needed information from the files.

The basic references come from the first 41 volumes of the Review of Applied Entomology, Series B. In addition, the card file has been checked against the Tropical Diseases Bulletin and many references have been added from individual papers encountered in the literature. The basic references are photographic prints of the abstracts and the reviews in the Review of Applied Entomology. These individual abstracts were cut and fastened to individual cards with Eastman's Tissuemat. Extra thin enlarging paper has kept down the bulk and the cards slide readily over one another, thus not hampering the needling processes. The Review, although exceedingly useful, has been rather disappointingly deficient in papers involving local distributions. Although the file has not been checked against the Zoological Record, there are about 40,000 cards in the file.

The cards in the files are separated into small categories thus only two of the categories have a rather unwieldy number of cards. The category for separating the cards is either by species of insects or by country. It is therefore rather easy to pick out the references for any given country or any given species.

The card used has sections for publication, date and author and 13 additional sections for the specific subject matter. More precisely, for the publication section with 499 categories, the literature in the field of medical entomology was reviewed and a list prepared of publications normally used in that field. The various publications are arranged alphabetically following the listings in the World List of Periodicals and are numbered for entry on the cards. The date section, with 99 categories provides only for the decade and the individual year. The section for author has 299 categories in which is used a standard author breakdown list of 299 categories. This has worked out very well because only rarely is there an unwieldy number of cards with more than one author for any given date and subject. The next 13 sections are identified on the card by alphabetical subscripts. Thus one may assign whatever kind of information to the section he wishes. To each section is assigned the special data to be itemized.

Section A with 499 categories is used for our list of countries of the world. Section B with 99 categories is used for entering the subdivisions of a country such as state or province. Papers on the faunal regions are indexed in section C with only nine numbered categories. Here all of the holes present are used so one can actually enter 15 categories. Section D with 99 categories is for season of activity of the adult arthropods. Sections E and F each with 99 categories are assigned to disease.

[1] Abstracted from a paper presented at the Fourth Annual Meeting of the Entomological Society of America held in New York City, December 27–30, 1956.

Here one may enter either two diseases, one in E and one in F, or one disease in E and something about the vector in F. Section G with 299 categories is for genus and here is used the same author breakdown code to designate author; but the code is so modified that an important genus such as *Anopheles* or *Culex* will be assigned its own individual number. Section H is for species, with 299 categories and here is used the author code unmodified. Although the author code was made for the names of people, it has worked satisfactorily for scientific names. Section I with 29 categories is used for breeding habitat. However, since any one paper may be concerned with several varieties of arthropods, in which case the section for breeding habitat cannot be used. To accommodate such papers Section I has a second meaning. This second meaning is used for those papers involving more than one kind of arthropod. Section J, K and L each with 99 categories are the general subject matter categories, with K being the most general of all. One might show in K that the paper is on ecology or epidemiology and then additional information can be placed in J and L. Section M with six direct code categories is used for century and other specialized uses that may occur from time to time.

Information is tabulated directly from the original articles and items entered on the sheets, such as species, season of activity (with a bar graph), data regarding the breeding habitats of the immature stages, and information concerning the habits of the adults that may be pertinent to the species and disease transmission and subdivision of the country in which the species occurs. Note is made whether the species is pestiferous or is a disease vector and finally the data are documented so that the responsibility for it can be traced to the author.

The first draft of the data from the Western Hemisphere is in typescript and data from 40 countries in the Australasian Region are about ready to type. In addition many tabulations for most of the countries of the world are in script in the files. As distributional information is encountered in the literature for different countries it is entered in the tabulations for that country. For instance, a paper on an arthropod group in Panama may have half the page space devoted to distributions in other parts of the world. And these distributions must be checked against the notations for each country.

It is hoped that some of the material may be published soon in some form so that it can be made available to the specialists of the world so they can help make the revisions that in time will make the list as accurate and as complete as possible.

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BOOK REVIEW

PHOSPHORUS AND FLUORINE, by B. C. Saunders. xv+231 pp. Cambridge University Press, London. 1957. $6.00.

This monograph is concerned mainly with phosphorofluoridates and fluoroacetates. Much of the described work arose as a consequence of investigations of war gases by the author and his colleagues at Cambridge during World War II. Although this is an interesting essay and not a catalog of facts, much data are presented from such relatively inaccessible literature as Ministry of Supply Reports, Reports of the British Intelligence Objectives Sub. Committee, and foreign patents. Subject matter is covered to 1955.

"Phosphorus and Fluorine" is written for advanced chemistry students and industrial chemists, but much of the material on anatomy, physiology and pharmacology is familiar to entomologists. Because the organic chemistry of phosphorus and fluorine has so many recent developments, the book begins with a general survey and a chapter on nomenclature of esters containing phosphorus. This should provide an adequate chemical background in this subject for entomologists. Most of the book describes the syntheses, reactions, biological effects, mode of action, biological uses, and analytical technics for organic compounds of fluorine and phosphorus. One chapter is a brief, lucid account of the mammalian nervous system. Another chapter, devoted to insecticides, emphasizes systemic action in plants.

Although it was shown in 1942 that esters of phosphorofluoridic acid inhibit cholinesterase and in 1947 that fluoroacetate blocked the oxidation of acetates, information on mode of action seems to have been of little use in planning their successful program. The author gives examples of tests on series of analogs and homologs of chemicals designed to find more active toxicants. Several attempts to combine biological effects of phosphorofluoridates with such toxins as fluoroacetates, mustard gas, triethyl lead, or phosphorodiamides are described. Examples of similar combinations are indicated for fluoroacetates. These combinations were attempted by syntheses of molecules containing both toxiphoric groups. The advantages of such combinations are obscure; it seems that this could be accomplished more easily by formulating mixtures so that each toxiphoric group could go to its site of action.

Fortunately, the biological effects of these materials have been employed for other than their original purpose. An interested reader can find many ideas for further work on relation of chemical structure to toxicity, mode of enzyme inhibition, antidotes, synergists, selective toxicity, and resistance mechanisms. Although the author often describes self-experimentation with toxicants, entomologists interested in safe handling of organophosphates will find helpful information in this book. If you work with untested fluorides or phosphates, read this, and recommend it to your physician.

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